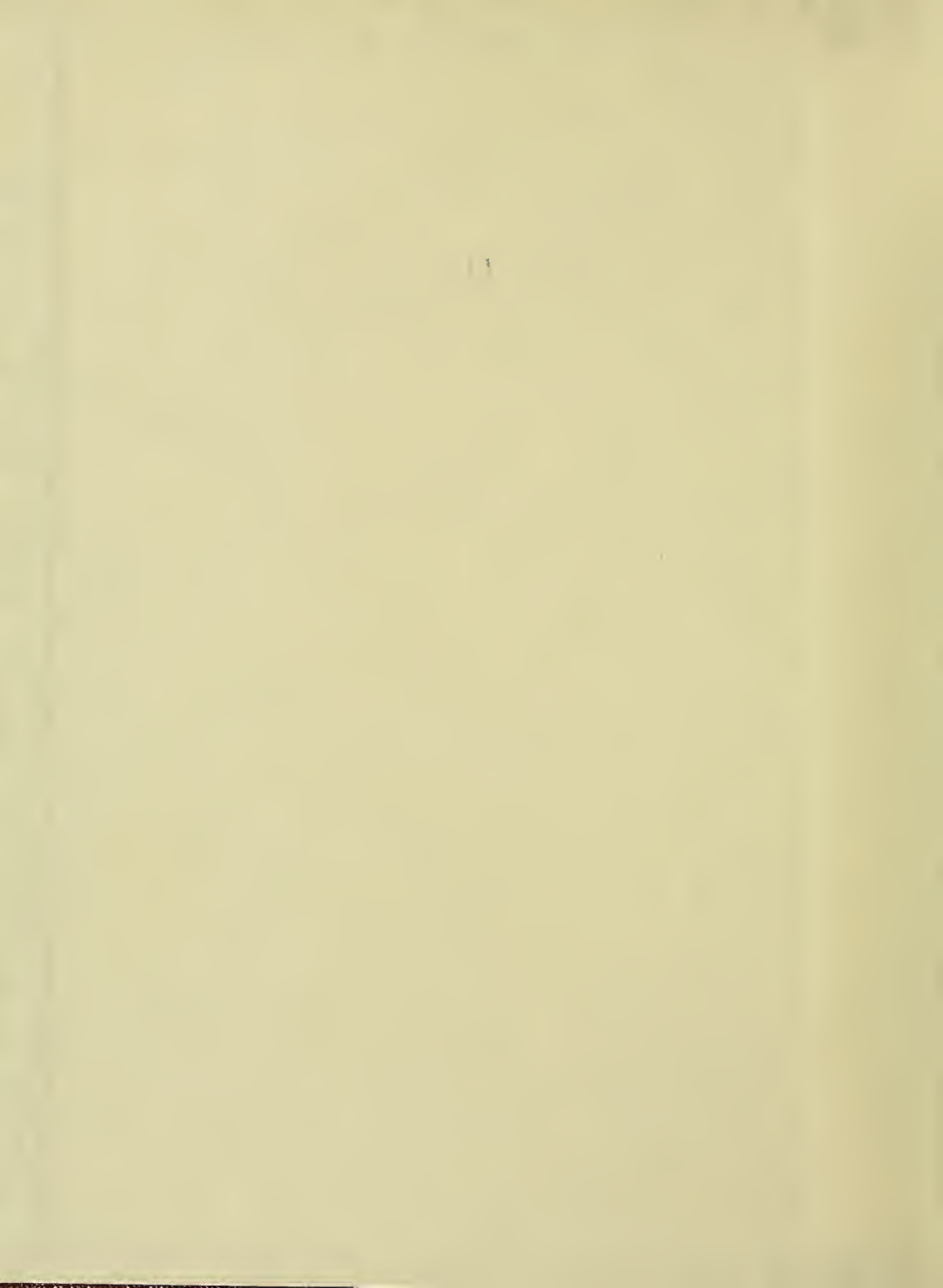
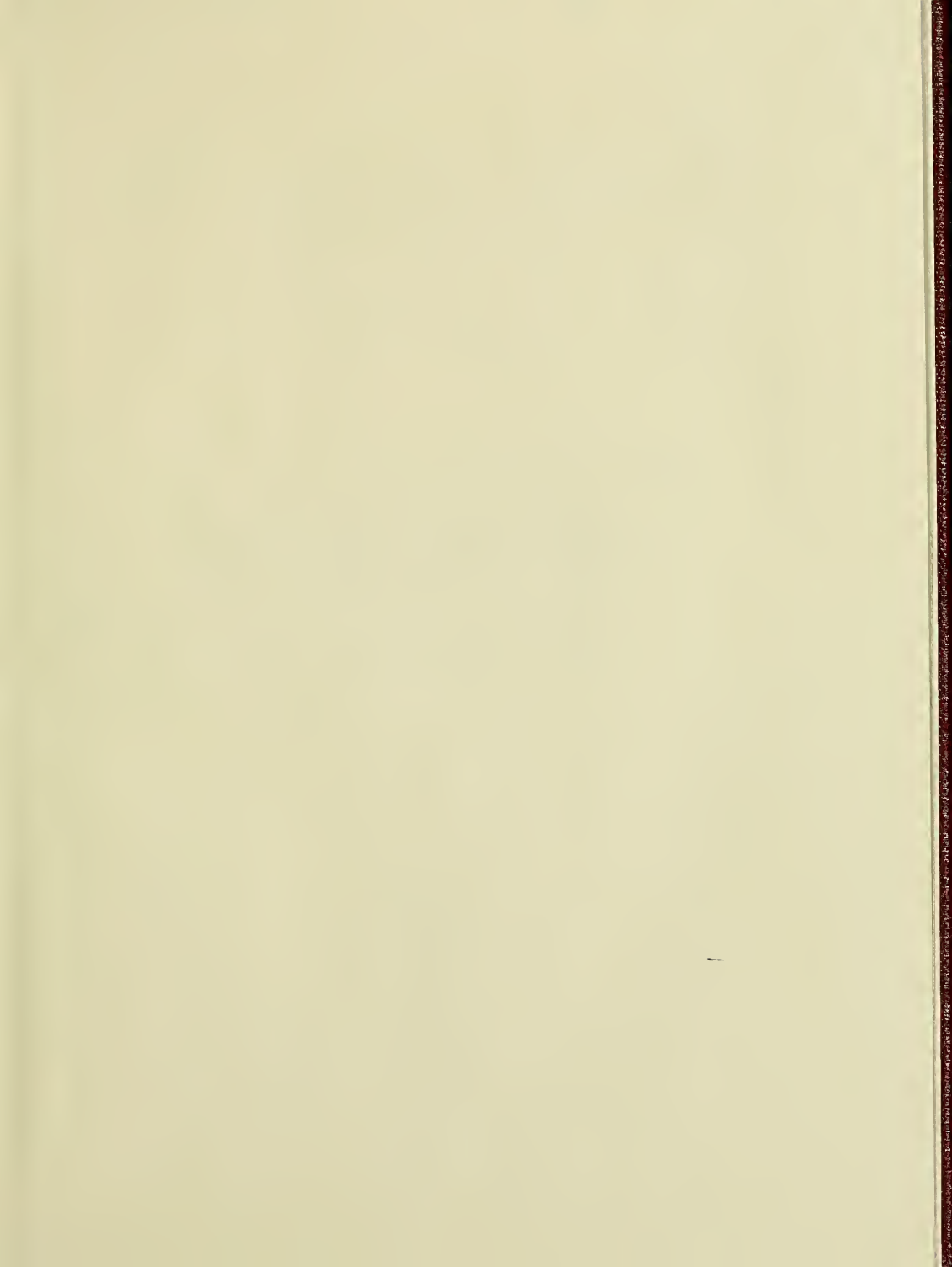


ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF MENTAL HEALTH
Fiscal Year 1980
Volume I

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health









ANNUAL REPORT *of program activities*

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

United States. NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1979 - September 30, 1980

VOLUME I

Summary Statements

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ANNUAL REPORT
MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research
NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1979 - September 30, 1980

TABLE OF CONTENTS

VOLUME I - SUMMARY STATEMENTS

	<u>Page</u>
Director, Mental Health Intramural Research Program.....	1
Director, Division of Clinical and Behavioral Research.....	7
Director, Division of Special Mental Health Research.....	11
 <u>DIVISION OF CLINICAL AND BEHAVIORAL RESEARCH</u>	
Biological Psychiatry Branch.....	13
Clinical Neuropharmacology Branch.....	21
Clinical Psychobiology Branch.....	25
Laboratory of Clinical Science.....	41
Laboratory of Developmental Psychology.....	57
Laboratory of Neuropsychology.....	65
Laboratory of Psychology and Psychopathology.....	83
Laboratory of Socio-Environmental Studies.....	99
 <u>DIVISION OF BIOLOGICAL AND BIOCHEMICAL RESEARCH</u>	
Laboratory of Brain Evolution and Behavior.....	109
Laboratory of Cerebral Metabolism.....	113
Laboratory of General and Comparative Biochemistry.....	117
Laboratory of Neurobiology.....	123

	<u>Page</u>
Laboratory of Neurochemistry.....	127
Laboratory of Neurophysiology.....	129
 <u>DIVISION OF SPECIAL MENTAL HEALTH RESEARCH</u>	
Laboratory of Clinical Psychopharmacology.....	143
Laboratory of Preclinical Pharmacology.....	147
 <u>Office of the Director - Intramural Research Program</u>	
Research Services Branch.....	161

ANNUAL REPORT
of the
DIRECTOR, MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1979 - September 30, 1980

John C. Eberhart, Ph.D.

These paragraphs introduce the annual report of the NIMH Intramural Research Program. The substance of the report is the summaries by the Laboratory and Branch Chiefs of the research of their units during the year ending September 30, 1980. The summaries follow this short piece and the comments by Dr. Robert Cohen, Deputy Director of this program.

A year ago and the year before that I devoted a good deal of this space to comments on the Civil Service Reform Act of 1978 and especially to the Senior Executive Service. The SES, together with the companion Merit Pay system, are the major innovations in personnel systems in recent years that affect the senior members of our staff. They are systems that are intended to improve government efficiency and productivity by giving more money for better staff performance, and some deprivations for poor performance. These "pay for performance" systems have had to be applied to scientists who already have a well developed reward system based on peer recognition and esteem, and which recognizes that the contributions by a scientist cannot be limited to or judged by any 12 month period, as the SES performance appraisal system purports to do.

The past year has really been the first operational year for the SES and almost so for the Merit Pay plan. A full account of the first year of the SES might be justified, but I am writing this well after the close of Fiscal Year 80 and will give only a brief summary.

The first comment is that the SES system, at least in this initial year, took a lot of time. Committee and task force meetings started it, and then there were training sessions for all concerned, the development of work plans for 16 SES members and their full discussion, the appraisal process itself for the same 16, laborious and touchy. Finally there were Performance Review Board meetings to review about 115 appraisals of intramural scientists at NIH, and the preparation and committee review of Presidential Rank nominations. It was and is a burden, about which one would not complain if he felt that the results were worth it. On that I remain skeptical.

The simplest summary of the first year is that we went through the prescribed routines without serious difficulty, executed all of the stages, and in the end, 4 SES members in the Intramural program received bonuses and 2 received Presidential Rank awards of Meritorious Executive. The system in general was stingy with its awards. Although the statute provided that bonuses could be awarded to as many as 50 percent of career SES positions, the Congress early in the year reduced this to 25 percent, and the Director of the Office of Personnel Management further reduced it to 20 percent of "eligible career employees." The

resentment among SES members at these restrictions was high. There were numerous cautions from OPM, Capitol Hill, and elsewhere to award bonuses with great discretion, to be sure nothing went wrong, to see to it that absolutely nothing bad happened on bonuses, because of what was perceived as limited and shallow support for bonuses in Congress, and the consequent possibility that Congress would knock out bonuses altogether. Such a step would have been welcomed by a good many in the SES.

The salary cap at \$50,012 stayed on for most in SES, and since bonuses were held down both as to number and amount the promised financial return for SES members was very disappointing. (On the amounts of the bonuses, the OPM ruled that no more than 5 percent of those receiving bonuses could receive the maximum, and that at least 70 percent of all bonuses awarded would be \$5,500 or less.) So that whereas supergrade employees offered positions in the SES had been told that 50% of SES members could get bonuses of as much as 20% of salary, when push came to shove only 5% of 20%, or 1% could get a bonus that high. Quite a change. It reminds me of the anecdote told by a Senate staff member to a seminar on Capitol Hill in March of 1981. To a Senator who had opposed many features of the CSRA when the bill was going through Congress, Alan Campbell, Director of OPM, said near the end of his stay that he was not sure that a pay-for-performance system was going to work. The Senator said if you weren't sure maybe we shouldn't have passed it. The Senator had argued against the system earlier, he said, because he knew that the Congress couldn't really manage a "performance-related" system in civil service. So far he seems to be more right than wrong.

As an indication that these views are not peculiar to me, let me quote from Secretary Richard Schweiker's testimony before the Senate Subcommittee on Civil Service, Post Office, and General Services of the Governmental Affairs Committee, on April 27, 1981. In testifying in support of the SES bonus system he said, in part, "The effect of these actions by Congress and the OPM [holding SES salaries to a fixed ceiling and limiting the number and size of bonuses] was to demoralize the majority of SES employees. Members of the SES had thought that they had a valid agreement with the Federal Government when they gave up their veterans preference and seniority rights and elected to join SES. Suddenly the rules were changed without Congressional hearings or the effort to obtain the views of the executives impacted by those actions. The reaction of most of those executives was that the Government had 'broken faith' with its managers.

"I wonder if it is apparent how compressed salaries have become. Although theoretically we have six pay rates in the SES, right now in HHS 518 of our 520 SES executives receive one rate of pay, \$50,112.50. The other two executives make \$52,750. Moreover, we have as many as seven successive levels of managers who all receive \$50,112.50. The high performance manager cannot be rewarded in such a system.

"A measure of the seriousness of the morale problem was reported on April 7 to the Subcommittee on Civil Service, House Committee on Post Office and Civil Service. The Federal Executive Institute Alumni Association reported the results of a recent survey of 228 SES members. When asked whether they would leave the SES if the option were available, 85% reported that they would."

On one other issue the year brought a discouraging clarity. The question had been, will the OPM admit to the SES a properly qualified GS-15 scientist without insisting that a nation-wide search be conducted for a candidate to fill a non-existent vacancy? The answer took a long time coming but it is now firm, and negative. OPM will not accept a justification based on "gradual assumption of duties" for approval of an SES appointment of a scientist. Scientists can still be proposed for genuine vacancies (a lab chief retires or resigns, leaving a real vacancy) and can win out in the subsequent competition, but we have lost the privilege of promoting a scientist from 15 to a supergrade position when the merits of his scientific achievements warrant it. That is a real loss.

I can't resist one paragraph on "performance appraisal," though the topic really requires more extended treatment. What concerns me here is appraisal of scientists though some of the same things could be said about appraisal of other staff. Dr. Cohen and I together interviewed and determined an appraisal on each of our 16 SES members. The special forms designed for the "Senior Scientific Service" spared us the crudity of having to assign numbers to indicate quality (or quantity?) of performance, as is required for other SES members, so we could choose between Outstanding, Above Average, Satisfactory, Minimally Satisfactory and Unsatisfactory. These would presumably be based on performance over the previous 12 months. As I had anticipated, there was no way I could truly rate the year's performance and ignore the previous 5, 10, or 20 years. We found it difficult to resist, and did not resist, the use of "outstanding" for outstanding scientists, despite some variations in achievements over the year. Within that category we had to make further distinctions, since we were permitted to recommend only 5 for bonuses, but we succeeded in doing that, not at all confident that we had made the right choices.

The performance appraisal was, in other words, an exercise, honestly tried, that seemed misdirected and unlikely to result in improved morale, greater effort, or improved confidence in management.

The story would not be complete without reporting that Presidential Rank Awards of Meritorious Executive were given to Dr. Julius Axelrod and the writer, and that bonus awards were given to Drs. Robert A. Cohen, Edward V. Evarts, Louis Sokoloff, and Marian R. Yarrow.

Administration

There were no major organizational changes in the Intramural Program during the year. For the record one laboratory was formally abolished in May, 1980, after it had been inactive and without staff for 3 years. It was the Laboratory of Neuropharmacology, last headed by Dr. Floyd Bloom. After Dr. Bloom and the other senior staff members left in 1975 - 77 we retained the structure but did not appoint new leadership, pending a review of the best use of the laboratory positions and budget. We eventually decided to terminate the Laboratory and use the resources to strengthen existing programs in the William A. White Building and on the Bethesda campus.

At about the same time we renamed the Laboratory of Clinical Psychopharmacology to better reflect its breadth of scope and its clinical research function. Its

new name is the Adult Psychiatry Branch, a term formerly used to designate what is now the Biological Psychiatry Branch. The new Adult Psychiatry Branch includes as one of its prominent components a Section on Aging.

During the year we finally completed a long but successful search for a new chief of the Laboratory of Psychology and Psychopathology, appointing to that post Dr. Allan F. Mirsky, Professor of Psychology in Psychiatry at Boston University School of Medicine. Dr. Mirsky came with a distinguished record of research and publications in cognitive and neuropsychology. Prior to his appointment at Boston he had been a member of the Psychology Laboratory staff here from 1954 to 1961.

A significant loss during the year was the retirement in December, 1979 of Dr. Harold Greenberg, long Chief of Clinical Care. Dr. Greenberg's contribution to the program in overseeing the care of patients and in helping to keep the welfare of patients as the highest value even in a research program was of great and lasting importance.

The Board of Scientific Counselors continued its faithful and critical review of the program in two meetings. On October 25-26, 1979, the Board reviewed the Laboratory of General and Comparative Biochemistry and the Laboratory of Neurochemistry. On March 27-28, 1980, the Board reviewed the Clinical Psychobiology Branch. On all three laboratories the Board reports were in general complimentary.

Awards

We were delighted at the election of Dr. Louis Sokoloff to the National Academy of Sciences this year, fitting recognition for his outstanding work in cerebral metabolism and neurochemistry over the years. Dr. Sokoloff was also honored by the F.O. Schmitt Prize for 1980, presented with the following citation:

"For his synthesis of neurochemistry, enzyme kinetics, and circulatory physiology in the development of techniques to quantitate cerebral metabolism and cerebral blood flow; for his use of these techniques to demonstrate the relations between glucose utilization and the functional activity of the brain and, in particular, to elucidate brain mechanisms of visual information processing, of psychotropic drug action, and of circadian rhythmicity; for his catalytic contributions to the work of other neuroscientists in their applications of his discoveries in clinical determinations of cerebral blood flow and metabolism by positron emission tomography; and for his pioneering work, which helped develop the field of modern neurochemistry."

Drs. Steven Paul and Paul Marangos won the A.E. Bennett Award for their paper on "The benzodiazepine-GABA-chloride ionophore receptor complex: common site of minor tranquilizer action."

Eight awards came to Intramural Program scientists this year from the Department and the Public Health Service. They were as follows:

Dr. Seymour Kaufman, the Department's Distinguished Service Award "for his fundamental contributions to neurochemistry and for extending that knowledge to the diagnosis and treatment of mentally-retarded victims or phenylketonuria and its variants."

Dr. Irwin J. Kopin, the Commissioned Corps Distinguished Service Medal "in recognition of his outstanding contributions as a clinical investigator, basic scientist, teacher, and scientific administrator."

Dr. Monte S. Buchsbaum, the Commissioned Corps Commendation Medal "in recognition of his pioneering role as a multidisciplinary researcher who combines electrophysiological, neurochemical, perceptual and cognitive measurements to attack problems of individual differences, psychiatric nosology and drug treatment response."

Dr. Elliot S. Gershon, the Commissioned Corps Commendation Medal "in recognition of his remarkable administrative and leadership ability and for developing new directions and new findings in biological genetic studies of psychiatric illness."

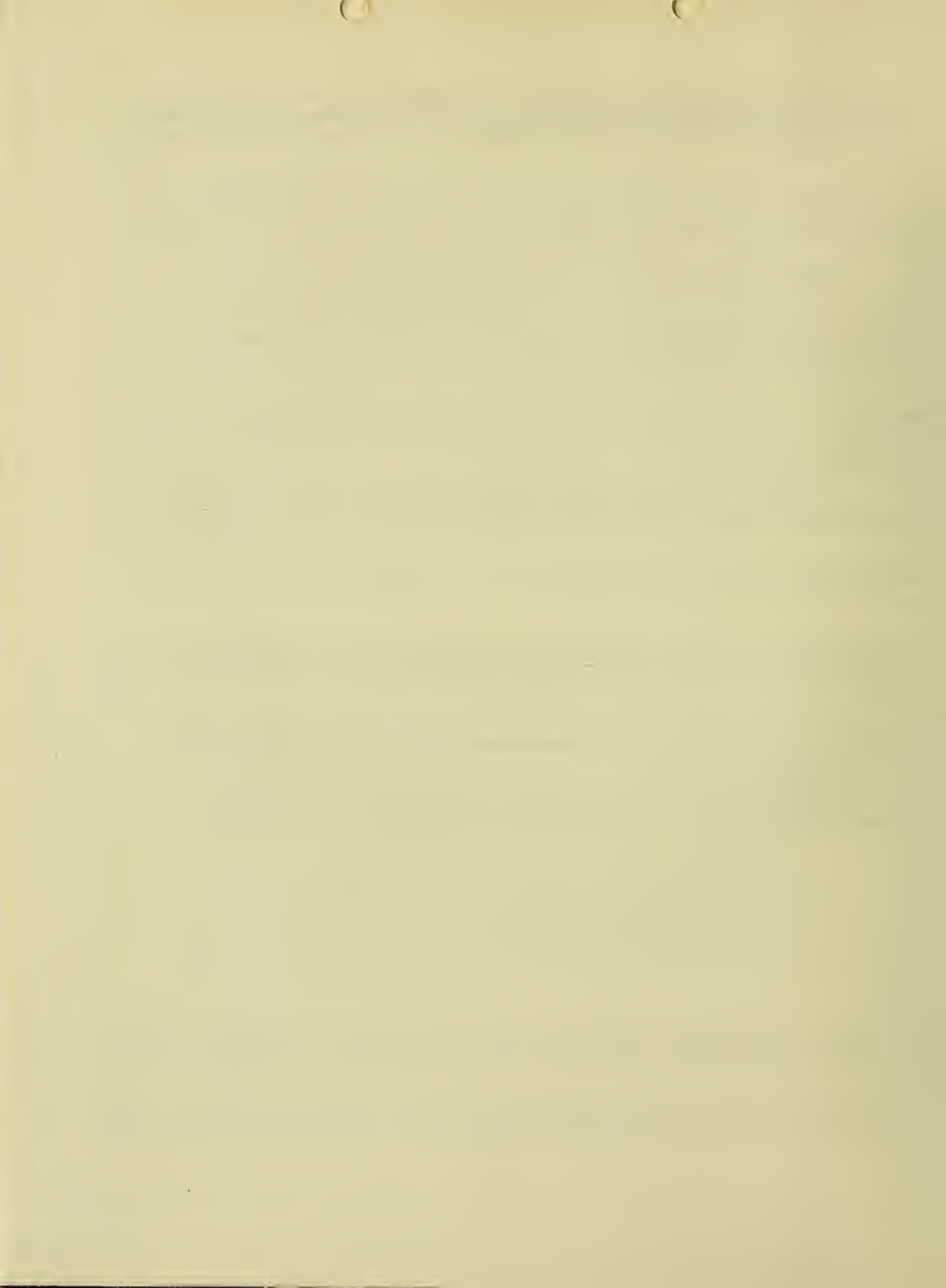
Dr. Frederick K. Goodwin, the PHS Superior Service Award "for his outstanding contributions to the study and treatment of the major psychoses."

Dr. Dennis L. Murphy, the PHS Superior Service Award "for his outstanding achievements as clinical investigator and team leader in research on the major affective disorders."

Dr. Richard J. Wyatt, the PHS Superior Service Award "for significant contributions to the understanding of the psychobiological processes in sleep and aging and the psychopathology of schizophrenia."

Mr. Newlin T. Morgan, the Commissioned Corps PHS Plaque "for sustained high quality performance of duty, exceptional dedication to the goals of the IRP, and exemplary demeanor."

It is these and many other distinctive contributions by NIMH scientists which account for the high quality of the Intramural Program.



Annual Report of the
Director, Division of Clinical and Behavioral Research
National Institute of Mental Health

October 1, 1979 - September 30, 1980

Robert A. Cohen, M.D., Ph.D.

As I read the reports of the Branch and Laboratory Chiefs, I am impressed again by the high quality maintained by the staff of this division. They work on important problems and are making significant progress. Professional awards and honors; invitations to address scientific meetings, to serve on committees and advisory boards which recommend science policy at national and even international levels have become so commonplace that they are now not even regularly mentioned. I am particularly pleased, also, that on July 1, 1980 Dr. Allan Mirsky returned to NIMH as Chief of the Laboratory of Psychology and Psychopathology. He was one of the first group of investigators brought together to form what is now the Laboratory of Neuropsychology. For over 20 years he has served as Professor of Psychology at Boston University Medical School. His recruitment was recommended by a search committee of extramural and intramural scientists which had reviewed the qualifications of several hundred research psychologists. His studies of attention will bring important new strengths to our program contributing, as they do, both to clinical problems and to a more fundamental understanding of central nervous system processes.

In sum, I think it fair to say that our work continues, essentially uninterrupted, at a level which is generally regarded as more than satisfactory. Yet my immediate office staff and I, as division director, have found this the most difficult of the 27 years the program has been in existence. We are literally deluged with requests for this bit of information or that, I must sign not one but two copies of innumerable sheets of paper, attend meetings to receive instructions as to new procedures to be followed and other meetings to certify that we have indeed met these requirements. In earlier days, it seemed to me that there were four essential elements in my job: first, to appoint good scientists; second, to provide administrative support that would facilitate their work; third, to ensure that the results of our studies were appropriately communicated; and fourth, to keep abreast of developments in fields relevant to our research. I have never worked in so unique a setting as NIH was at that time. There were few obligations which were not clearly relevant to the achievement of these goals.

But now NIH and NIMH have changed; the pressing needs of society today seem more complex than they were a quarter century ago. Accountability and evaluation are essential and vital for a nation which finds its resources finite and its needs infinite. It is only a slight exaggeration to say that this year I have felt more like a certified public accountant than a science administrator. It is not that the requests made of us or that the new regulations we must observe are unreasonable. In some instances they may

even lead to desirable change. But, in my view, it is their universal application which may have unfortunate effects. It is as if the dietetic department decreed that every patient in the hospital must be given exactly the same diet--a diet which on the basis of careful research was found to contain just the amount of nutrients, vitamins, minerals and roughage which would maintain an average healthy individual in an excellent nutritional state. I need not detail the havoc such a decree would bring about.

The Senior Scientific Service, to which we have devoted a significant amount of time is a case in point. Ours is an egalitarian society, but it has never been claimed that the manager of every large enterprise should receive the same salary as those at each operating level of the organization. In the Federal Government generally there has been a steadily increasing pressure at the top salary levels; presently those with the greatest responsibility receive the same salary as others with considerably less and, in some instances, because of special inducements to professions difficult to recruit, supervisors may be paid less than those they supervise. For years, many research associates and perhaps the majority of clinical associates have left NIH for university posts which pay higher salaries than are received by the laboratory chiefs who supervised their research training. Those senior staff members who, for whatever reasons, could no longer tolerate this state of affairs went on to university positions themselves. Our success in keeping others was at least partly due to our ability to distribute scarce resources in ways which enabled dedicated investigators to push forward in their work, to exploit their findings in important new areas.

The Senior Scientific Service promised, for a price, to correct some of the salary imbalance. Few creative scientists have any cause to be concerned about Civil Service or any other tenure. And since the so-called annual inflation adjustments are almost never given to those at the top salary levels anyway, most of us gave up our tenure and joined the system. Congress then cut the proposed number of awards by 60%; this did not take anything away from us, but understandably increased cynicism to a degree. Meanwhile, we pressed forward with our definition of the critical elements and major responsibilities of our jobs and, later, with the evaluation of our accomplishments. The effort we put forth was sincere, but the results were not meaningful. Perhaps with continued review and with exchange of ideas and experiences we will improve. But for the present, at least, the business school method of evaluating productivity in an automobile factory has not been usefully adapted to the evaluation of creativity in a biomedical research institute. Our definitions of responsibilities and work plans did not reflect the highly individual ways in which creative research ideas come to mind and are carried out.

One final point: all of us were gratified when John Eberhart and Julius Axelrod received the award of Meritorious Executive. Eberhart heads and wisely governs a psychiatric research program which is widely recognized as having made more substantive contributions and having trained more leading investigators than any other in the world. Axelrod's current research is at least as exciting as the earlier work which won him the Nobel Prize; thirty of the forty research associates who were trained in his laboratory already hold major professorial posts; last year, the work of only one

other scientist (in any field) was quoted in scientific journals more frequently than his. Since none of us belongs to the school which believes that only those who die heroic deaths should be eligible for the Congressional Medal of Honor, our pleasure at their awards was tempered by our curiosity about just what it takes to be considered for the higher level of Distinguished Executive.

For these reasons, among others, I do not yet find that the Senior Scientific Service or any of the other new systems and regulations under which we operate contribute positively more than they complicate our lives. Perhaps, the reader will be as amused as I by Wellington's letter to British Army Headquarters written when he was on campaign in Spain during the Napoleonic Wars. The letter was quoted in a talk by Dr. Sherman M. Mellinkoff, Professor of Medicine and Dean of the Medical School of the University of California at Los Angeles.

"Gentlemen:

"Whilst marching to Portugal to a position which commands the approach to Madrid and the French forces, my officers have been diligently complying with your requests which have been sent to H.M. ship from London to Lisbon and then by dispatch rider to our headquarters.

"We have enumerated our saddles, bridles, tents and tent poles, and all manner of sundry items for which His Majesty's Government holds me accountable. I have dispatched reports on the character, wit and spleen of every officer. Each item and every farthing has been accounted for, with two regrettable exceptions for which I beg your indulgence.

"Unfortunately, the sum of one shilling and ninepence remains unaccounted for in one infantry battalion's petty cash and there has been a hideous confusion as to the numbers of jars of raspberry jam issued to one cavalry regiment during a sandstorm in western Spain. This reprehensive carelessness may be related to the pressure of circumstances since we are at war with France, a fact which may come as a bit of a surprise to you gentlemen in Whitehall.

"This brings me to my present purpose, which is to request elucidation of my instructions from His Majesty's Government, so that I may better understand why I am dragging an army over these barren plains. I construe that perforce it must be one of two alternative duties, as given below. I shall pursue either one with the best of my ability but I cannot do both:

"1. To train an army of uniformed British clerks in Spain for the benefit of the accountants and copy-boys in London, or perchance,

"2. To see to it that the forces of Napoleon are driven out of Spain.

Your most obedient servant,
Wellington"

We know, of course, that the rules and regulations of the War Office did not prevent Wellington from defeating Napoleon at Waterloo, nor did they arrest the growth of the Empire. As I remarked in the beginning, the growing set of rules and regulations under which we labor have not yet adversely affected our scientific work. From one point of view, one can consider them a small price to pay for continued public support of science. Certainly they were not instituted only for the purpose of establishing closer and more critical surveillance of government-supported research. Rather, they are manifestations of a general attitude prevailing in current culture which as citizens we may share, but of which as scientists we become the object. Individual segments of society are no longer given sole responsibility for assuring that they operate effectively for the public good. It remains to be seen whether the methods prescribed for the conduct of our program and its evaluation accomplish their purpose without impairing sensitive institutional supports for creativity.

Annual Report of the
Director, Division of Special Mental Health Research
National Institute of Mental Health
October 1, 1979 - September 30, 1980

Richard Jed Wyatt, M.D.

As I write this report again, the research program of the Mental Health Institute has completed its 23rd year in the William Allison White Building. It is pleasing to report that this has been another fine year for the Division. What is most on my mind at this moment is the duality of scientific enterprise--it is both strong and fragile. Since the Middle Ages, science has progressed exponentially--slowly, at first, but at a visibly rapid pace over the last hundred years.

When this Division began, all computations were done either by hand, on slide rules, or on mechanical calculators. Today, more computing power will fit in my palm than could be generated by our store room full of antique computers. Not only do we use the computer for computations, but every new piece of equipment we purchase is guided in some way by a microprocessor.

The power of science is awesome. This year the World Health Organization announced the worldwide eradication of small pox. Numerous groups are approaching the practical use of recombinant DNA. Yet, scientific investigations are also very vulnerable. Just a few years ago, China appeared to be regressing into its own middle age, with a governmental decision that scientific pursuits were not important. Now, however, governmental policy has changed and streams of Chinese scholars are coming through our laboratories and we are invited to their's.

The scientific effort of our Division is thriving. Unfortunately, increasing amounts of paper work, hiring freezes, uncertainty, mistrust of government, and the extraordinary inflation throughout the economy have taken their toll. In addition is a continual need to modify our research plans as new techniques develop. Where once a row of cabinets, table tops and sinks served as a laboratory, we now are installing special environmentally controlled rooms for tissue culture. While just a few years ago special rooms had to be constructed for our electrophysiology experiments, modern electronics make them no longer necessary, and in fact, the heavy shieldings are a hindrance.

Over the course of the next year an unusual amount of construction will continue or begin at William Allison White Building. We have almost completed a new photography and graphic arts laboratory, the installation of a building-wide fire alarm and have just finished the first stage of a security system, enclosing the perimeter of the building. We have started to remodel the first floor laboratories, space for the day hospital, and our conference room. The new chief (when chosen) for the section in the director's office (to replace the abolished Neuropharmacology Laboratory) will surely need to remodel space for his or her needs.

Our scientific activities are many and are detailed in the project reports. Several projects seem especially worthy of mention because they may have immediate clinical importance. Drs. Neff and Lackovic have discovered that dopamine and its metabolites are present in peripheral nerves of the rat, cow, and human. It appears that dopamine may function as a neurotransmitter in these nerves, perhaps playing a role in cardiovascular and urogenital function. Almost certainly these dopaminergic nerves play a role in some of the peripheral manifestations of parkinsonism and in some of the side effects of neuroleptic drugs which block dopaminergic function. Drs. Schwartz and Breakefield (Department of Medical Genetics, Yale University) have demonstrated that nerve growth factor from dysautonomic patients, while normal in quantity, is biologically inactive. Since one of the principal manifestations of familial dysautonomia is the dysfunction of the sympathetic nervous system and nerve growth factor is necessary for sympathetic nerve development, this abnormality could be related to the cause of the disorder. A few years ago, Dr. Carman noted that psychiatric patients lost weight when they received the hormone calcitonin. Following up on this observation, Dr. Freed demonstrated in rats that calcitonin, which is secreted into the blood stream after a meal, produces satiety through a direct action on the brain. Dr. Freed is currently measuring calcitonin in patients with anorexia nervosa to determine whether an excess of calcitonin might be responsible for some anorexics' inability to eat.

The members of our staff continue to merit high recognition. Dr. Sitaram, working under Dr. Gillin in the Sleep Laboratory, received the A.E. Bennett Award for Biological Psychiatry; I was given the Public Health Service Superior Achievement Award; and Dr. Costa was decorated with the Cavaliere Officiate by the President of the Italian Republic.

Having begun this report on things both strong and fragile, let me close on a similar note. We greatly regret the passing of Leah Sanford, a trusted and dedicated member of our staff for many years.

ANNUAL REPORT OF THE BIOLOGICAL PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1979 - September 30, 1980

William E. Bunney, Jr., M.D.

During the past year the Branch has continued its productivity in terms of papers published and invited lectureships. On March 29-30, 1979, the Branch was reviewed by the Board of Scientific Counselors of the NIMH. In their report they stated, "the overall impression of the research of the Branch was highly favorable".

In recognition of his remarkable administrative and leadership ability, and for developing new directions and new findings in biological genetic studies of psychiatric illness, Dr. Elliot Gershon received the Commendation Award of the U.S. Public Health Service.

The Branch continues to play an active role in the Collaborative Studies in Biological Psychiatry of the World Health Organization. The Branch was the leading center for a project on red-green color blindness as a genetic marker which was coordinated by Dr. Elliot Gershon and the final paper was published in 1979. The Branch is also a lead center for a study on the therapeutic efficacy of naloxone in mania and schizophrenia. Data for this project is in the final stages of analysis. Data analysis suggests that there is a significant decrease in specific rating items in schizophrenic patients following administration of naloxone.

Many of the research activities of the eight sections or units of the BPB are reviewed below.

Unit on Childhood Mental Illness

This unit focuses on an investigation of biological factors in behaviorally disturbed pediatric populations and on those at risk for later psychiatric disorders, primarily hyperactive children and offspring of parents with affective disorders.

A series of pharmacokinetic studies have demonstrated that a clinical response to amphetamine in hyperactive children occurs primarily during the absorption phase rather than the elimination phase of drug metabolism. This implies that a saturation of receptors, rather than plasma concentration per se, may be important for clinical response.

One of the leading hypotheses concerning the etiology of hyperactivity in children suggests that the functional levels of the neurotransmitter dopamine may be a relevant factor. A negative therapeutic study with L-DOPA, a precursor of norepinephrine and dopamine and ET-495 or piribedil, a dopamine agonist, are clearly not supportive of this important hypothesis.

Behavioral studies ongoing in the unit indicate that morning stimulant drug doses decrease daytime activity but may increase evening activity, a

"rebound effect" that may have adverse significance for treatment and which has never, to our knowledge, been described before in the literature.

Another study indicates that the offspring of affectively disturbed patients show a significantly greater incidence of depression than do offspring of normal controls, but that children of medically ill controls fall between these two groups. This finding supports both genetic and environmental contributions to childhood depression. A study of lithium treatment in behaviorally disturbed offspring of lithium-responding parents indicates that a variety of behavioral disturbances in addition to manic-depressive illness of children, may be responsive to lithium therapy.

Ongoing studies were conducted to examine acute and sub-acute effects of caffeine in normal boys. Acute effects (10 mg/kg) were increased restlessness and decreased reaction time. Chronic effects, including symptoms of caffeineism (headache, insomnia, restlessness), were noted by mothers.

It is anticipated that in the near future this unit will be designated a section with Dr. Judith Rapoport as its Section Chief.

Section on Neuropsychopharmacology

This Section is involved in the study of schizophrenia from a clinical, biochemical and pharmacological perspective. The report by Wagemaker and Cade that hemodialysis leads to significant clinical improvement of schizophrenia in open trials has attracted wide public and scientific interest and has led numerous groups of psychiatrists and nephrologists throughout the world to investigate these claims, while many other groups have already begun to use hemodialysis as a treatment without any scientific evidence of its efficacy. For this reason it was felt very important to conduct a double-blind sham-dialysis-controlled study. Final results of this study included eight chronic schizophrenic patients who completed a protocol consisting of ten weekly sessions of active dialysis and ten weekly sessions of sham dialysis. None of the patients improved during active dialysis, while four patients became worse. Thus, previous reports of therapeutic efficacy were not replicated in this investigation. Although the possibility of a small subgroup of dialysis-responsive schizophrenic patients remains, our data indicates that hemodialysis cannot be considered to be a treatment for schizophrenia at this time.

A major hypothesis in schizophrenia which is under investigation throughout the world suggests that functional levels of dopamine may be increased. Amphetamine has a number of actions including the release of dopamine. Studies in this section have shown that chronic neuroleptic treatment did not block behavioral activating effects but did prevent the effects of amphetamine on sleep. Lithium did not prevent sleep effects of amphetamine but did attenuate activation and euphoria. Furthermore, it was observed that response to amphetamine during the period after withdrawal of pimozide did not indicate a dopamine receptor supersensitivity, as has been hypothesized by many scientists. It was noted that lithium has mild anti-psychotic and anti-depressant properties in schizophrenic patients.

A study testing the potential efficacy of short periods of treatment with beta-endorphin produced only minimal behavioral effects and no evidence of therapeutic efficacy. Studies of CSF in schizophrenic patients reported increased CSF-norepinephrine, decreased gamma-amino-butyric acid, dopamine-beta-hydroxylase and the pteridine cofactor.

Section on Psychogenetics

The Section on Psychogenetics has as its goals: 1) identification of biological and psychological factors that are genetically transmitted in major psychiatric disorders and, 2) pharmacological and pharmacogenetic studies of psychoactive agents in well state patients and in normal controls.

In studies of the pharmacogenetics of psychoactive drugs, genetic control of vulnerability in behavioral and physiological effects of d-amphetamine have been identified in normal volunteer twins. Furthermore, behavioral responses, prolactin and growth hormone release after amphetamine are highly heritable. Ninety percent of the variance in behavioral excitation response to amphetamine can be predicted from baseline measures of 3-methoxy-4-hydroxyphenylglycol (MHPG) and prolactin in plasma.

Studies of amphetamine response in patients off medication are nearing completion and data analysis has now begun.

In genetic biologic studies of psychiatric disorders, the family study of primary affective disorders has reached the stage of completed data collection and review. Schizoaffective patients have the highest proportion of illness in relatives followed by bipolar patients and then unipolar patients. Genetically, bipolar I and II patients appear equivalent. A significant rate of illness is present in spouses of patients, implying assortative mating.

Genetic analysis of patients in whom CSF metabolites have been studied revealed that patients with positive family histories of alcoholism and sociopathy have significantly lower baseline levels of homovanillic acid (HVA), MHPG, and norepinephrine (NE).

The Section sponsored a major conference on Genetic Research Strategies for Psychobiology and Psychiatry. The scientific goals of the conference were: a) to consider new techniques applicable to genetic questions in psychobiology and psychiatry and, b) to develop the use of genetic techniques to unravel regulatory mechanisms and establish relationships among psychobiological phenomena. The major idea to come out of the conference was that cellular and molecular genetics can now contribute greatly to progress in psychiatry and psychobiology. Current feasible uses of recombinant DNA technology in psychobiology are critical. Concomitant with these developments, cell culture can be used to isolate single chromosomes in somatic cell hybrids and to study physiologic alterations produced by presence or absence of specific chromosomes or DNA fragments.

It is a specific goal of this Section of the Branch to develop the laboratory and personnel capacity to take advantage of these new techniques. It is our belief that these scientific areas constitute the leading edge in psychogenetics and therefore it is critical to develop such a capacity.

Section on Clinical Psychophysiology

A major current focus of this Section is on a new program of collaborative research on local cortical area functioning using EEG and positron emission tomography. A first patient has been studied using this important new technique, 18F-2DG positron emission tomography. This technique allows three-dimensional visualization of the metabolic activity of the brain.

In addition, this Section is currently exploring the use of multichannel electrophysiological maps as an adjunctive procedure. Initial pioneering studies look very encouraging. Thus researchers in this Section will be utilizing 16 to 64 EEG leads recorded during 2DG uptake. This data can currently be displayed on maps of cortical glucose use.

In addition to this, using somatosensory evoked potentials (EPs) and signal detection psychophysics, this Section, in collaboration with the Unit on Drug Abuse, was the first to report a hyperalgesic effect of the opiate blocker naloxone in man. In controlled and double-blind studies they noted that morphine produced the opposite of the specific evoked potential augmenting effect caused by naloxone.

Unit on Sleep Studies

Continued evidence has been derived in this Section to support the hypothesis that patients with primary affective illness, both in remission and while ill, exhibit cholinergic muscarinic supersensitivity. It was shown that a faster response on the cholinergic REM Induction Test occurred in patients who were depressed and in remission, in contrast to normals. In addition, these patients showed greater pupillary constriction to pilocarpine, a muscarinic agonist, which is also compatible with cholinergic muscarinic supersensitivity. It has also been hypothesized that these abnormalities may reflect an underlying phase advance of circadian rhythms. In collaboration with Drs. Weingartner and Ebert, this Section has shown evidence suggesting that lecithin may be helpful in the cognitive deficits of Alzheimer's disease.

A major study of the clinical benefits and risks associated with flurazepam in insomniac patients has been completed. Drs. Gillin and Mendelson are serving on the Intergovernmental Coordinating Committee of the Surgeon General's Project on Sleep.

Unit on Studies of Drug Abuse

The study of the endogenous opioid peptides (endorphins), their clinical neurobiology and their contributions to human behavior and psychiatric illness is a major research goal of this Unit. Clinical strategies include the

assessment of behavioral effects accompanying the narcotic antagonist naloxone and beta-endorphin administrations to psychiatric patients. The development of a radioreceptor assay for opioid activity in human CSF and plasma has provided a tool to study biochemical aspects of the endorphin system functioning in vivo, including the effects of psychopharmacologic agents and drugs of abuse with mood altering properties.

The Unit has completed a study of the behavioral and biological effects of intravenous administration of beta-endorphin to psychiatric patients. While there were no significant group effects observed for the schizophrenic or depressed patients, three individual schizophrenic patients showed apparent worsening.

In collaboration with Dr. Candace Pert, the Unit has successfully developed a radioreceptor assay for determining plasma and CSF opioid activity in man. It was found in normal volunteers that plasma opioid activity appears to be secreted episodically and that there is a diurnal rhythm with elevated morning levels. This rhythm is similar to that of ACTH and may have relevance to our finding of decreased pain sensitivity in morning hours. It was also found that plasma opioid activity was significantly elevated during mania in comparison to depression in one cycling manic-depressive patient. CSF opioid activity has been determined in a large number of psychiatric patients and this data is currently being analyzed.

Section on Biochemistry and Pharmacology

Studies in this section are directed toward the understanding of how the brain can modulate the sensitivity of its response to neurotransmitters.

Beta-adrenergic receptors in cultured HeLa and astrocytoma cells continue to be studied with the idea of having cell culture systems where receptor induction may be changed by altering the media and culture conditions. It has been shown that butyrate treatment can cause an increase in beta-receptors in the absence of any increase in catecholamine-activated adenylyl cyclase. This demonstrates that the "coupling" is a separate event from the increase in beta-adrenergic receptor number. Phospholipid synthesis is under beta-adrenergic control and an increase in phosphatidylcholine synthesis occurs in the presence of beta-agonists. In turn, phospholipids control the number of adrenergic receptors and turnover of phospholipids may be an initial step in desensitization.

It has been demonstrated that there is an interaction between the GABAergic system and the mechanism of action of the minor tranquilizers. It has been shown that there is a high affinity site in the brain which seems to be the site of action of diazepam and that the affinity of this site for diazepam may be increased by the addition of GABA. Chloride and other halides also enhance [³H]diazepam binding. These effects may be related to a postulated chloride ionophore controlled by GABA. This Section has clearly shown that the activation of the GABAergic system renders the brain more sensitive to benzodiazepines. It is thus possible that benzodiazepines alter neuronal inhibition in the CNS and that this may be one of their modes of action.

Similarly, the anticonvulsant agent diphenylhydantoin has been shown by investigation in this Section to increase [³H]diazepam binding by unmasking cryptic sites in brain. This increase in the number of binding sites is accompanied by profound changes in the electrophysiological response to the benzodiazepines and this may be important in attempting to understand the anticonvulsant properties of dilantin. Additionally, pretreatment of pregnant rats with dilantin leads to alterations postnatally which have been correlated to altered susceptibility to seizures in children whose mothers have received anticonvulsant therapy.

The alpha-adrenergic receptor was measured in the rat superior cervical ganglion. The location of alpha-receptors in the sympathetic ganglion and their function in transmission through the ganglion are being studied. New peptide hormone receptors for vasoactive intestinal polypeptide (VIP) and bombesin are being studied in brain. The distribution and immunoreactivity of bombesin-like material suggests the possibility that bombesin may be a transmitter. Also, an increase in beta-endorphin has been observed in female rats in labor. Behavioral studies have been conducted that show that descending noradrenergic and serotonergic pathways appear to mediate beta-endorphin analgesia. Work continues to show that one of the possible modes of action of lithium is its ability to alter neuronal receptor function.

Section on Psychobiology

Ongoing studies of the temporal lobe-limbic anticonvulsant, carbamazepine, continue to document its acute efficacy in more than half of the manic patients and slightly less of the depressed patients studied to date. In addition, preliminary evidence suggests that carbamazepine may have prophylactic effects against recurrences of both manic and depressive episodes. These acute and prophylactic findings are of particular interest, since they are observed in patients, many of whom are treatment resistant and have a history of non-response to lithium carbonate. Thus these findings suggest that carbamazepine may be a useful alternative treatment to lithium.

Work continues in the study of the neurotransmitter and peptide effects of carbamazepine in order to acquire knowledge concerning its possible modes of action. CSF, but not urinary MHPG, is significantly reduced during carbamazepine treatment, while probenecid-induced accumulations of the homovanillic acid (HVA) metabolite of dopamine also appear decreased. At the same time there is evidence that carbamazepine increases prolactin, suggesting that the drug has complicated effects on dopamine metabolism that are not consistent with either simple dopamine agonist or antagonist function. There is no significant effect of carbamazepine on CSF GABA OR CSF opiate binding activities. The latter findings are of interest in relation to carbamazepine's clinical utility in a variety of pain syndromes and reports in animals of its potentiating opiate hyperactivity.

Using recent methodological advances it has been documented that the NE metabolite MHPG in plasma is significantly higher in manic than in depressive phases of patients with rapidly cycling manic-depressive illness. These data,

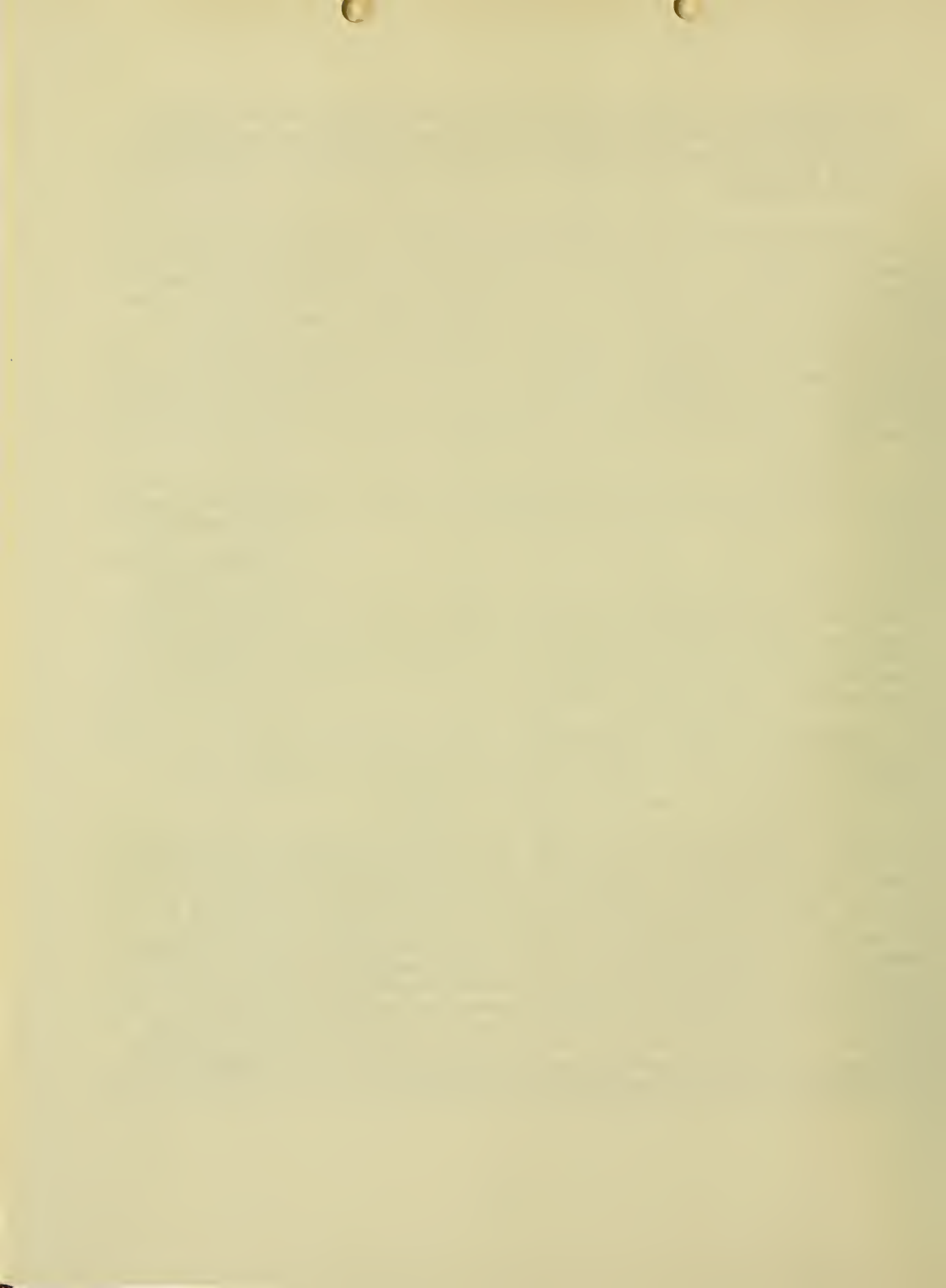
taken in conjunction with similar findings of urinary MHPG and the observations of increased CSF NE in mania, are consistent with the notion of different levels of noradrenergic function in the manic compared to the depressed phase of the illness, even though values are not always different from those in normal volunteers.

For the first time it has been documented that the phenomenon of tardive dyskinesia may be closely associated with mood state in patients with manic-depressive illness. In two rapidly cycling manic-depressive patients, exacerbations of tardive dyskinesia were observed during the depressive phase of the illness with dramatic improvement during mania. These observations are not easily compatible with current theories suggesting dopaminergic receptor hypersensitivity in tardive dyskinesia and excess dopaminergic activity in mania compared to depression. The findings of marked exacerbation of tardive dyskinesia during depressed compared to manic phases suggest that one or both of these current leading theories of dopaminergic mechanisms in tardive dyskinesia or affective illness may require reconsideration.

The Section has documented significant elevations in urinary free cortisol in bipolar compared with unipolar depressed patients and decreases in manic patients compared with normal volunteers. Urinary free cortisol appears to be closely associated with psychological state and, in some instances, cortisol elevations appear to precede the behavioral switch into depression. Marked alterations in the pattern of cortisol secretion have also been documented in 20-minute plasma samples in patients studied longitudinally before, during, and after sleep deprivation-induced mood improvement and, in one patient, during a spontaneous switch into hypomania. These studies confirm earlier reports of cortisol hypersecretion and lack of diurnal variation. In addition they note for the first time absence of sleep-related growth hormone peaks and a growth hormone dysrhythmia in depression.

A systematic method of charting the entire life course of affective illness has been developed. Evidence from these studies are consistent with a variety of evidence that there is a tendency for faster recurrence of each affective episode as a function of number of episodes.

Work is continuing to examine the interrelationship between electrophysiological and pharmacological kindling, as well as the effects of these chronic treatments on brain functional behavior. Repeated administration of subconvulsive doses of the local anesthetic lidocaine is eventually associated with the appearance of major motor seizures of increasing severity and duration. It has been documented that these seizures are selectively activated in limbic system structures, particularly the hippocampus, amygdala, as well as cingulate gyrus, as documented by increases in metabolic activity measured by the ^{14}C -2-deoxyglucose method of Sokoloff, Kennedy and associates. Repeated activation of these limbic seizures with lidocaine is associated with marked increases in aggressive behavior. Thus this pharmacological kindling paradigm may be a useful model for the dissection of the relationship of progressive behavioral alterations in repeated limbic seizures.



Annual Report of the Clinical Neuropharmacology Branch
National Institute of Mental Health
October 1, 1979 to September 30, 1980

Dennis L. Murphy, M.D., Chief

Over the last year, the Clinical Neuropharmacology Branch developed several major new areas of research effort, established four research administrative subunits within the Branch, moved into its new clinical research unit on 6D in the NIH Clinical Center, moved all of its offices (the second time in two years), and sponsored a series of symposia at national and international meetings summing up research findings in two of its long-standing research areas, monoamine oxidase and monoamine oxidase-inhibiting drugs, and the platelet model for studies of neurotransmitter function.

The major focus of the Branch remains the same--the evaluation of psychopharmacologic agents in man with the aims of better understanding their mechanisms of action and of elucidating, through study of their effects, the contributions of the brain neurotransmitter systems to behavior. As before, most of our research efforts are concentrated on studies conducted on our clinical research unit, with a great deal of emphasis on collaborative studies done with individuals outside the Branch who have expertise in such areas as the study of sleep and other psychophysiologic phenomena, memory function, and the quantitation of neurotransmitter metabolites and hormones in human plasma, urine and cerebrospinal fluid. Our own limited laboratory space and animal work are also used to support and supplement the clinical studies.

In moving into the 6D clinical research unit in January, 1980, we gained a nicely renovated but substantially smaller center for our group's activities. 6D is especially cramped in space for daily patient activities, but the many hours put into design of the unit paid off in ensuring reasonable clinical and research operations in its restricted space. Overall responsibility for the 6D inpatient studies was assumed by Robert M. Cohen, M.D., Ph.D., chief of the unit on clinical pharmacology. During the past year, the clinical studies on 3-East and subsequently on 6D were very ably served by Larry Siever, M.D., and Ned Kalin, M.D., who were the ward administrators on the research units.

At the end of the year Larry Siever assumed overall responsibility for the expanding area of outpatient studies in the Branch, and became chief of the new unit on biological correlates of behavior. The Branch's outpatient studies have been principally directed towards investigations of possible vulnerability factors for psychopathology among non-patient, community samples, employing the biological high-risk strategy. Carol Hoover, D.S.W., became chief of the unit on family assessment. Involvement of family members in the treatment program has continued to prove an important ingredient both in the treatment of severely depressed patients and as an important source of information for the research work.

Some new study areas developed over the past year include a project, under the direction of Thomas Insel, M.D., which is devoted to the investigation and treatment of severe obsessive-compulsive individuals. This disorder has been very little studied from a psychobiologic and psychopharmacologic standpoint, but represents an often severe, chronic and treatment-resistant problem among the major psychiatric disorders. Many of the initial referrals to this project were obtained as a result of adult inquiries regarding Dr. Rapoport's project on obsessive-compulsive disorders in children carried out in the NIMH Biological Psychiatry Branch.

Other new projects include the development of several neuroendocrinologic investigations using different neurotransmitter-related agents to investigate brain-behavior relationships. Dr. Siever is using clonidine as an α -adrenergic agonist, and Drs. Risch, Kalin and Cohen have been investigating the cholinergic agonists physostigmine and arecholine, as well as some possible neurotransmitter mechanisms underlying the phenomenon of CNS control of dexamethasone suppression of ACTH and cortisol. All of these neurotransmitter-neuroendocrine relationships are believed to be altered in individuals with affective disorders. Specific findings include the delineation of a circadian rhythm for cortisol in cerebrospinal fluid and evidence that among the various neuroendocrine alterations accompanying physostigmine administration, the magnitude of change in plasma β -endorphin correlates most highly with the behavioral and affective changes produced by this cholinergic agent. While β -endorphin and ACTH release are believed to be under similar regulatory control, plasma cortisol concentration changes did not correlate with physostigmine's behavioral effects. In addition, dexamethasone was found to suppress only plasma cortisol but not plasma β -endorphin concentrations in man--providing further evidence for a possible dissociation of these neuroendocrine control mechanisms in man.

In the laboratory, Dr. Cohen has been responsible for the establishment of a series of assays for neurohormones and neuropeptides which have been used in our clinical studies. Dr. Kalin has been developing the Branch's non-human primate studies program, and has improved the methodology for obtaining 24-hour collections of cerebrospinal fluid for the measurement of various hormones, amines and amine metabolites. The departure of Dr. Iain Campbell and the inability to refill a biochemist support position in the laboratory for the entire year because of the hiring freeze reduced the amount of work accomplished in the area of investigations of amine metabolism and monoamine oxidase in the last year.

In Dr. Jonathan Costa's laboratory studies, the mechanisms involved in amine storage in human platelets have been defined by a series of investigations employing labeled amines, nuclear-magnetic resonance spectroscopy and energy-loss spectroscopy. Human amine storage vesicles differ from pig amine storage vesicles in containing more calcium and pyrophosphate and less magnesium. Possibly because of these compositional differences, the maximal capacity for storage of serotonin, dopamine and quinine is approximately three times greater in human compared to pig platelets. The amine uptake rate is also substantially greater, as is the maximal release of vesicles produced by calcium ionophores or thrombin. Amines in

vesicles do not appear to form simple macromolecular complexes, but in each species the stored amines appear to assume the particular molecular properties of its vesicular compartments. Similar mechanisms for amine storage have been suggested for other amine-containing cells in neurons, but have proved to be more difficult to study than in the platelet model.

In laboratory and animal studies of monoamine oxidase (MAO), large primate-rodent species differences in the proportion of the MAO subtypes MAO-A and MAO-B were demonstrated. Brain tissue from three primate species (human, rhesus and vervet) all possessed substantially higher proportions of MAO-B than MAO-A activity across different brain regions, while rats, mice and hamsters all had higher MAO-A activity. These differences raise questions about the applicability of rodent models in studies of some aspects of brain amine metabolism and amine-affecting drugs, as an increasing number of widely used drugs including the tricyclic antidepressants and the amphetamines have been shown to have differential inhibitory effects on the two enzyme subtypes.

Studies of platelet monoamine oxidase activity as a possible reflector of biological vulnerability to psychopathology and as a correlate of various normal behaviors have been continued in normal populations. In an 18-month follow-up investigation of individuals originally identified as having extremes of low or high platelet MAO activity and further subdivided according to cortical average evoked potential measurements, considerably higher incidences of hypomanic and depressive episodes meeting RDC diagnostic criteria were found in the low MAO/augmenting and high MAO/reducing subjects. These same subgroups also had been found to be associated with affective psychopathology in the original retrospective study.

In other studies, platelet MAO activity differences were found to be associated with an elevated risk for the development of behavioral side effects in alcoholic individuals receiving disulfiram. Also, reduced platelet MAO activity has been found to be associated with elevated plasma prolactin levels and with increased eye blink rates in schizophrenic patients. While it has been hypothesized that such differences might reflect alteration in brain rather than peripheral MAO activity in these individuals, no direct evidence in support of this possibility has been forthcoming. However, several studies to explore brain-platelet MAO relationships have been instituted in non-human primates, particularly vervet and rhesus species. Fairly strong associations between platelet MAO activity and social behavior were identified in a study in rhesus monkeys which was published last year. Symposia at several national meetings were organized last year on biological vulnerability factors and also on studies of selective MAO inhibitors. In addition, an international meeting which was cosponsored on monoamine oxidase in 1979 led to the publication this year of a book, "Monoamine Oxidase: Structure, Function and Altered Functions."

Further evaluation of data from our completed study comparing a selective inhibitor of MAO-A (clorgyline) with a moderately selective inhibitor of MAO-B (pargyline) in psychiatric patients led to some additional conclusions not originally appreciated. It now appears that

clorgyline (the most therapeutically effective of these two structurally-similar drugs) possesses antianxiety and antidysphoria actions equal to its antidepressant effects. Clorgyline also appeared to be particularly effective in the more severely depressed and endogenous-type patients, in contrast to most other clinically-available MAO-inhibiting drugs, which have generally been found most helpful in "atypical" depression subgroups. The antidepressant and antianxiety effects of clorgyline correlated most highly with changes in norepinephrine concentrations in cerebrospinal fluid and plasma, rather than with changes in amine metabolites in cerebrospinal fluid, including 5-hydroxyindoleacetic acid, homovanillic acid or 3-methoxy, 4-hydroxy phenylglycol. Enhanced sensitivity to the pressor effects of tyramine after clorgyline and pargyline administrations was found to be highly correlated with plasma 3-methoxy, 4-hydroxy phenylglycol concentration reductions, and to be more altered by clorgyline than pargyline, suggesting that norepinephrine changes are also of major importance in this potentially dangerous side effect of MAO inhibitor treatment.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Frederick K. Goodwin, M.D.

The Clinical Psychobiology Branch continues to direct its primary research effort toward understanding the spectrum of affective disorders. Our fundamental assumption is that alterations in mood and behavior are expressions of the complex interrelationships between biology, and environment; we have therefore emphasized the development of a series of interrelated biological studies of circadian rhythms, monoamine neurotransmitter systems, neuroendocrine systems, neurophysiology and pharmacokinetics.

One of the major strengths of the Branch has been its ability to pursue these interrelated clinical studies together with related basic research. Thus, our clinical studies of circadian rhythms in affective illness have been enriched by animal studies of the effects of psychotropic agents on biological rhythms in animals. Human studies of the effects of antidepressants on cerebrospinal fluid monoamine metabolites provide confirmation of observed in vitro effects of the drugs. Animal studies of central nervous system receptors for benzodiazepines and tricyclic antidepressants help elucidate the mechanisms of action of these important psychotropic drugs.

In addition to conducting basic research which is directly relevant to our clinical interests, the Branch also conducts research in fundamental brain neurochemistry (such as the identification and characterization of neuron-specific enolases) that promises to advance our knowledge of such basic central nervous system processes as neuronal differentiation and energy generation; the potential clinical relevance of such basic research is only now becoming apparent.

The ability of the Branch to pursue this diverse, yet interrelated set of research projects has depended primarily on our capacity to recruit investigators whose training and interests span both clinical and basic science. This year both a new Clinical Associate (Dr. Becker) and a PRAT Fellow (Dr. Hauger) have joined the Branch; they will work initially in the area clinical inpatient research. The Branch has been fortunate to have both Dr. Rehavi (a Chaim Weizmann Post-Doctoral Fellow from Israel) and Dr. Hays (an NIMH Post-Doctoral Fellow) working on studies of receptor sites in the central nervous system. Dr. Crawley, a PRAT Fellow, is developing novel animal behavior models for the evaluation of drug effects. She and Dr. Ross, also a PRAT Fellow, will be developing our capacity for basic neurophysiological research. Dr. Kitanaka, a Fogarty Visiting Fellow, will be working with Dr. Potter on pharmacokinetic studies. Through an IPA agreement with Duke University, Dr. Linnoila will pursue a variety of clinical research projects in both inpatient and outpatient settings, focussing on clinical pharmacology and neuroendocrinology. Finally, a major expansion of our capacity for outpatient studies is now under way, with the addition of Drs. Thompson (a Clinical Associate) and Gardner (a psychiatric resident on a half-time IPA appointment) to the Outpatient Unit staff.

PROJECT SUMMARIES

The following summaries reflect the major research projects of the Branch during the past year. They are described in greater detail in the accompanying project reports.

The Clinical Research Unit is a thirteen bed inpatient unit devoted to the study of major affective disorders. This unit is the primary focus for the clinical research of the Branch, it provides comprehensive psychiatric care for patients involved in our research projects. Under Dr. Wehr's direction, the Unit screens and admits patients, gathers general psychiatric and psychosocial information, and coordinates the efforts of the Branch's researchers to provide an appropriate evaluation and treatment program geared to the clinical needs of the patient and the research interests of the Unit.

Traditionally, the emphasis of our specialized resources has been the longitudinal study of episodes of affective illness, before, during and following a variety of therapeutic interventions. Initially, studies focused on classical, discrete episodes of mania or endogenous depression; more recently, our inpatient resources have been devoted increasingly to individuals with rapid-cycling bipolar illness. During the past year, a new dimension has been added to the inpatient studies, with the introduction of a "brief inpatient evaluation" program for depression, in which outpatients are admitted to the Unit for one week of pre-treatment studies and are then discharged to be treated as outpatients (see Project Z01 MH 01852-02). This approach enables us to evaluate a much broader spectrum of depressive disorders, using the methodologies previously applied to the endogenous depressions, without seriously compromising our ongoing longitudinal studies of cycling bipolar illness.

An important facet of our work is the continuous collection of essential data, that can then be used as a part of specific research projects. Thus, behavior and mood are regularly rated by patients and nursing staff; blood and urine collections are scheduled on a regular basis; motor behavior is continuously monitored by solid-state activity monitors; and sleep is recorded nightly. Within this general framework, specific evaluation and treatment protocols are introduced. Evaluation protocols include detailed studies of circadian rhythms, neuroendocrine challenge tests, sleep recordings using the electroencephalogram (EEG), and cerebrospinal fluid collections. Some treatment approaches are non-pharmacologic, such as sleep shifts and sleep deprivation. Pharmacologic studies include a crossover trial of desmethyl-imipramine and zimelidine (permitting clinical and biochemical comparisons of a noradrenergic tricyclic antidepressant with a serotonergic tetracyclic antidepressant), a trial of propranolol in mania, and a variety of treatments for rapid-cycling bipolar illness.

The current plans for the Clinical Research Unit emphasize continuing longitudinal study of rapid cycling illness, a new project to study biological effects of electroconvulsive therapy (ECT), and an expansion of the brief inpatient evaluation program.

Finally, to provide a control population for our research in affective illness, the Unit admits and studies "normal volunteers", using many of the research paradigms applied to affectively ill patients.

Biological rhythms in affective illness have become a major focus of both clinical research and animal research. Dr. Thomas Wehr directs this research. Our basic hypothesis is that bipolar illness is itself a recurrent and in some cases clearly a cyclical illness, that disturbances in circadian rhythms are demonstrable during episodes of illness, and that the disturbances in these circadian rhythms are closely related to the pathophysiology of affective episodes. Thus, this project focuses on identifying alterations in circadian rhythms of activity, rapid eye movement (REM) sleep, temperature, neuroendocrine function, and neurotransmitter activity, and then associating these alterations with altered clinical states. Several technological advances have greatly facilitated this endeavor. A new generation self-contained, solid-state activity monitor developed here at NIH permits the continuous recording of activity. This small device is worn on the wrist and records total activity at 15 minute intervals, storing more than one week's data. This enables extensive outpatient as well as inpatient studies. More recently temperature data has been simultaneously obtained using a rectal thermister and similar solid-state technology. Either a blood pump with an indwelling catheter or a heparin lock with interval sampling is used to produce samples for analysis of circadian rhythms of substances in blood.

Major findings this year include the following:

In depression, the timing of circadian rhythms is abnormally early (phase-advanced) relative to the day-night cycle when compared with controls. The circadian rhythm of urinary MHPG excretion is phase-advanced, but to a lesser degree. The night-time peak of melatonin secretion is advanced approximately one hour.

In rapid-cycling bipolar patients, the temperature rhythm shifts with the changes in mood, and is maximally phase-advanced at the time of the switch into depression.

The phase-advance seen in depressed patients may be simulated in normals by shifting their sleep period later in the night. With such a shift, the REM sleep patterns of normals resemble the REM sleep patterns of depressed patients. Analysis of the effects of such sleep shifts on temperature and neuroendocrine rhythms are under way.

Shifting the sleep period of depressed patients earlier has been associated with a remission of the depression in some patients, an effect presumably related to the alteration in the phase-relationships of circadian rhythms.

The switch into mania is accompanied by alternate nights of sleeplessness in over 50% of all bipolar patients, suggesting a 48-hour sleep-wake cycle rather than the usual 24-hour cycle. Such clinical observations are consistent with a model of mania which hypothesizes a marked slowing of the sleep-wake cycle, such that it "escapes" from its normal

entrainment to other 24-hour rhythms and instead becomes coupled with these rhythms on alternate days, producing a 48-hour cycle. This 48-hour day is also similar to the pattern of activity occurring in humans isolated from external time cues in caves or other similar settings, in whom similar uncoupling of circadian rhythms occurs. Thus, the 48-hour days in early mania either may indicate a marked slowing of the oscillator controlling the sleep-wake cycle, or may indicate a subsensitivity in mania to the daily external stimuli such as light that function as time cues to entrain activity to a 24-hour cycle.

A study by Drs. Wehr and Lewy found that the suppression of night-time melatonin secretion by intense light is intact in patients, suggesting that there is no gross subsensitivity to the external entraining cue of light in depression.

Studies of the effects of psychotropic drugs on circadian rhythms in animals have been performed by Dr. Wirz-Justice, with Dr. Wehr and other collaborators. In animals entrained to a 24-hour light-dark cycle, imipramine and clorgyline induce 4-12 hour delays in the phase positions of the circadian rhythms of several receptors (the circadian receptor rhythms are noted in the number of available receptors). In free-running hamsters, both imipramine and clorgyline slow the activity-rest cycle, an effect which could be expressed as a delay of the phase position of the rest-activity cycle if it were entrained to a 24-hour light-dark cycle. Research is planned to investigate whether other cycles (such as receptor binding) are also slowed under free-running conditions. These results are consistent with the hypotheses that depressed patients exhibit an advanced rest-activity cycle relative to entraining cues, and that antidepressants act by slowing the intrinsic rhythm of certain circadian rhythms, such as the rest-activity cycle.

A comprehensive and critical review of the literature revealed remarkably consistent findings of a phase-advance of a wide variety of circadian rhythms in depression.

The phase-advance model of depression can account for the REM sleep disturbances reported in endogenous depression.

Melatonin studies in humans have been greatly facilitated by the development by Drs. Lewy and Markey of a highly sensitive and specific gas chromatographic-mass spectrometric (GC-MS) assay. This assay enables us to conduct clinical research on the circadian pattern of melatonin secretion by the pineal gland and its alteration in depression and other disease states. In addition, our melatonin assay permits a reliable examination of the effects of drugs and of light on melatonin secretion. Finally, since melatonin secretion is largely regulated by beta noradrenergic mechanisms, melatonin levels can be used for in vivo assessment of pre- and post-synaptic beta-adrenergic functioning.

Major findings this year include:

Preliminary findings of differences in melatonin secretion in affectively-ill patients compared with controls have been confirmed. In normals, night-time melatonin levels rise to a peak of 25-100 picograms/ml prior to dawn, and fall rapidly thereafter to very low daytime levels. Melatonin secretion is increased in manic patients and decreased in depressed patients. This is consistent with the basic formulation of the catecholamine hypothesis (which posits an increase in noradrenergic activity in mania and a decrease in depression) since the secretion of melatonin reflects the activity of β -adrenergic receptors in the pineal.

Blind individuals, studied collaboratively with Drs. Ballantine and Newsome (NEI), have grossly abnormal secretory patterns of melatonin.

We have previously reported that artificial light of normal intensity does not suppress night-time melatonin secretion in humans, unlike the response in animals. We have now shown a dose-response relationship between light intensity and suppression, with intensities intermediate between the intensity of normal artificial light and the intensity of sunlight producing intermediate degrees of suppression. This methodology enables us to compare the dose-response curve in affectively-ill patients with that in normal controls.

Contrary to our preliminary findings, there appears to be no correlation between REM sleep and melatonin secretion.

Animal studies of melatonin secretion suggest that chronic treatment with desmethylinipramine (DMI) attenuates the isoproterenol-induced increase in melatonin by 20%, suggesting a reduction in sensitivity of the post-synaptic beta-adrenergic receptor. However, since the naturally occurring peak of melatonin is reduced by 50% during DMI administration, some additional alteration of pre-synaptic functioning is implied. Data analysis of DMI effects on human melatonin secretion in normals and affectively-ill patients is under way.

Amine neurotransmitters and metabolites have continued to play a significant role in the overall research program of the Branch. The Clinical Psychobiology Branch serves as the coordinating unit for cerebrospinal fluid (CSF) studies in the Division, and works collaboratively with units which provide assay capabilities, such as the Laboratory of Clinical Science. Urinary 3-methoxy-4-hydroxyphenylglycol (MHPG) is assayed in our laboratory.

A variety of methodological issues have been central to our concerns this past year. Several completed studies suggest that the administration of probenecid to block egress of acid neurotransmitter metabolites from CSF may introduce major new sources of confounding variance: the incidence of nausea and vomiting is related to CSF probenecid levels and is independent of dose; weight changes during hospitalization affect CSF probenecid levels; CSF metabolite accumulation is significantly correlated with CSF probenecid

levels, but the strength of the correlation varies among diagnostic groups; and medications such as lithium affect the CSF metabolite-probenecid correlation. Since the GC-MS assay enables sensitive and specific determination of low levels of metabolites, our focus has shifted away from the use of probenecid.

This year, the Branch has placed a high priority on the development of high pressure liquid chromatographic (HPLC) methods for assaying CSF metabolites. When fully developed and verified against the GC-MS, the HPLC methods should provide sensitive, accurate assays, available in our own laboratory.

A major methodological investigation of assays for urinary MHPG has been completed. The current modified GC assay for MHPG correlates highly ($r = .98$) with a GC-MS assay, but the absolute levels are about 20% higher. Thus, the two methods each produce highly reliable results, but results across assays may not be entirely comparable.

Significant findings this year include the following:

The effects on monoamine metabolites of potentially confounding variables such as age, sex, and activity must be assessed separately within each diagnostic group. Age, sex, and activity are significantly related to urinary MHPG levels only in depressed patients, not in normals. CSF probenecid levels are significantly correlated with CSF metabolite levels in bipolar patients, less highly correlated in unipolar patients, and not correlated in schizophrenics. Whether these varying relationships are of pathophysiological significance remains to be determined.

Extensions of prior studies found no relationship between urinary MHPG and either CSF or plasma MHPG, suggesting that MHPG in different body fluids reflects different aspects of noradrenergic function.

A replication of previous urinary MHPG studies in affective illness reconfirms earlier findings: urinary MHPG is significantly lower in depression than in mania, and is significantly lower in depressed bipolar patients than in either depressed unipolar patients or normals.

Patients with primary affective disorder who have a family history of alcoholism (corresponding to Winokur's depression spectrum illness) were found to have lower CSF 5-hydroxyindoleacetic acid (5-HIAA) than those without a family history of alcoholism. This finding may relate to earlier studies from our lab which suggested an association between serotonergic mechanisms and alcoholism.

Chronic desmethylimipramine selectively decreased CSF MHPG and had little effect on CSF 5-HIAA, whereas zimelidine decreased CSF 5-HIAA and had little effect on CSF MHPG. Both drugs decreased urinary MHPG excretion, as previously noted. In addition to the suggestion that CSF MHPG may correlate more closely with drug effects than urinary MHPG, the results indicate that non-probenecid LP's may be as useful as

probenecid LP's in identifying drug effects. One further finding of interest is that the degree of change in metabolite levels bore no relationship to either global clinical response or to drug effects on circadian patterns of motor activity.

Two studies using the probenecid method to assess metabolite changes induced by ECT and lithium suggest that these treatments produce similar changes in CSF metabolites. CSF 5-HIAA is significantly decreased by both treatments; however, neither initial 5-HIAA levels nor the decrease in 5-HIAA were related to clinical response. CSF HVA was not significantly altered by either treatment. There was, however, a strong relationship between pretreatment HVA levels and subsequent changes during treatment, with high HVA levels decreasing and low HVA levels increasing. However, again neither the pretreatment levels nor the changes during treatment were related to clinical response.

The availability of a brief inpatient evaluation program will allow us to examine monoamine metabolites in a broader spectrum of patients. Of particular interest is whether patients with non-endogenous depressive syndromes have high urinary MHPG (as suggested by Schildkraut) and whether individuals with disorders of affect and impulse control (borderline personalities) demonstrate low CSF 5-HIAA (as suggested by our previous studies of sociopaths).

Dr. William Potter coordinates the Branch's research involving the Clinical Pharmacology of Antidepressants. Three distinct areas have been pursued: (1) the development of reliable assays for antidepressants, (2) the use of pharmacokinetics to provide maximally effective use of drugs, and (3) the study of clinical and biochemical effects of specific antidepressants in patients and normals as a means of studying the biochemistry of major affective disorders.

Studies of the pharmacokinetics of the tricyclic antidepressants (TCA's) have relied on highly sensitive and specific, but time- and equipment-intensive GC-MS assays. This past year, we have established and validated a high pressure liquid chromatographic (HPLC) assay for TCA's and their hydroxylated metabolites.

Pharmacokinetic studies provide a means of studying crucial variables in drug response by assessing absorption, distribution, and metabolism of the drug, and by relating steady-state levels of the drug to observed biological and behavioral effects. Important findings this past year include the following:

Single dose administration of DMI with subsequent measurement of plasma drug levels accurately predicts the dosage required to achieve satisfactory "steady-state" plasma levels with daily drug administration.

Although there is significant interindividual variation in rates of metabolism, we found that drug metabolism in nursing home patients is indistinguishable from metabolism in both a somewhat younger patient group and a group of normal volunteers in their early 20's.

Studies of the hydroxylated metabolite of TCA's suggest that it is pharmacologically active, has a very short half-life, and that about 5% of the population are essentially non-hydroxylators (a fact of considerable pharmacogenetic interest).

Studies of CSF levels of TCA's and tetracyclic antidepressants suggest that CSF levels provide a useful measure of free (and thus biologically active) drug.

Ten patients have now completed a unique double-blind cross-over trial in which an experimental antidepressant (zimelidine), specific for serotonin uptake inhibition, is compared with an established antidepressant (desipramine) specific for norepinephrine uptake inhibition. The preliminary finding that zimelidine does not precipitate mania or rapid cycles in bipolar depressed patients (as contrasted to desipramine) has been extended; in addition we have provided further evidence of a lack of overall difference in the antidepressant efficacy of the two drugs, despite differential effects on MHPG and 5-HIAA in the CSF.

In volunteers the effects of desipramine on urinary and plasma MHPG and plasma NE are consistent with a primary drug action as an inhibitor of the reuptake of released NE: NE itself is increased, while its deaminated metabolites are decreased. These data do not provide support for the assumption that decreases in MHPG reflect decreased turnover of NE.

Following acute administration of DMI, normal controls show decreases in plasma growth hormone (GH), while depressed individuals show no GH change. This differential result is similar to that observed after amphetamine administration.

Both DMI and zimelidine produced similar and marked effects in the rest-activity cycle of depressed patients independent of their effect on mood. With both drugs, total activity increased while "night-time" activity (midnight to 7 a.m.) dramatically decreased.

Neuroendocrine studies of major psychiatric disorders have continued under the direction of Dr. Philip Gold. These studies receive impetus from many directions: first, several aspects of the symptom complex of the functional psychoses suggest hypothalamic dysfunction which may be reflected in neuroendocrine abnormalities; second, monoamine neurotransmitters seem to modulate synthesis and release of many hypothalamic peptides and pituitary hormones, suggesting that neuroendocrine function may provide a window into central monoaminergic systems; and finally, neuroendocrine peptides and hormones may themselves have direct behavioral effects.

Studies of monoaminergically regulated hypothalamic-pituitary systems have produced the following results:

Findings that neuroendocrine responses to dopamine infusions are abnormal in both schizophrenia and affective disorders have been extended in larger groups of patients. While normal subjects show suppression of plasma LH during infusion followed by LH rebound, schizophrenics show enhanced suppression with no rebound and manics show a paradoxical rise in LH during infusion.

Using the "integrated neuroendocrine procedure", unipolar patients show enhanced GH and prolactin responses to arginine (consistent with augmented serotonergic function), while manic patients show enhanced LH response to LHRH (consistent with augmented endogenous LHRH activity, which may be clinically reflected by increased libido).

Urinary free cortisol (UFC) is significantly elevated in psychotic schizophrenics, as it is in psychotic depressed patients.

In rapid cycling bipolar patients, UFC is high in depression and lower in mania. In some patients, the UFC excretion shifts prior to the mood switch from one state into the other.

A major area of new research involves behaviorally active central nervous system (CNS) peptides.

The role of the CNS peptide vasopressin (VP) is currently under study, particularly in patients with affective illness. In a major published review, we formulated an hypothesis that suggests a role for vasopressin in the regulation of affect in man. The review stressed the functional relationship between vasopressin and many aspects of the symptom complex of affective illness, including alterations in the consolidation of memory, modulation of pain sensitivity, synchronization of biological rhythms, timing and quality of REM sleep, and the regulation of fluid and electrolyte balance.

We have now demonstrated that CSF VP is significantly elevated in mania and significantly decreased in depression, when compared with normal controls. Enhanced VP response to an infusion of hypertonic saline in manic patients also suggests augmented VP function in mania.

In preliminary studies, a VP analog, DDAVP, induced highly significant and consistent improvements in tests assessing the formation, encoding, and organization of long-term memory traces. These improvements occur both in depressed patients and in normal volunteers. In some depressed patients, improvement in depressive and psychotic symptoms also occurred.

Studies of VP in anorexia nervosa, in collaboration with Drs. Kaye and Ebert of the Laboratory of Clinical Science, demonstrate marked state independent increases in the ratio of CSF VP to plasma VP, and marked variations in VP response during the administration of hypertonic saline.

CSF somatostatin levels are high in mania and low in depression compared with normals. There is a non-significant trend for levels to be lower in bipolar than in unipolar depressed patients.

Neuroendocrine response to opiate agonists and antagonists have been evaluated. Naloxone administration has little effect on a variety of neuroendocrine parameters. In contrast, administration of methadone or beta-endorphin produces a marked rise in prolactin, a moderate rise in growth hormone, and a marked suppression of cortisol. The cortisol suppression after methadone is long-lived, suggesting that a methadone-suppression test (analogous to the dexamethasone suppression test) may help evaluate the set-point for opiate inhibition of the hypothalamic-pituitary-adrenal axis.

Both acute and chronic DMI administration significantly augment prolactin levels in depressed patients. The significance of these findings in terms of possible effects of DMI on prolactin-dependent breast tumors remains to be elucidated.

Outpatient followup studies of manic depressive patients and families have been conducted by Ms. Davenport and Dr. Cowdry. These studies involve 60-70 outpatients with bipolar affective disorder who are seen in the clinic on a weekly or monthly basis. Most are maintained on lithium. A variety of psychotherapeutic modalities are used, with the primary emphasis on group therapy and crisis intervention.

It is well-known that lithium maintenance is not always effective in preventing a recurrence of bipolar illness. Recurrences may be associated with specific biological features of the illness (such as rapid cycling) with specific psychosocial stresses, or poor compliance with recommended treatment. A study completed this year found that factors associated with good compliance with prescribed medication or scheduled appointments include: being married, perceiving the clinic as providing continuity of care, and having no significant periods of elevated mood.

Recurrences may also occur in association with childbirth. Two of three bipolar women maintained off lithium through pregnancy developed post-partum manias. One of three bipolar men studied through their normal wife's pregnancy also developed a mania. A retrospective record review suggests that over 50% of the men in the clinic have had at least one episode in close contiguity to a child's birth.

The six infants born to these parents have been studied in collaboration with Drs. Cytryn, Lamour, Yarrow, and Waxler using a standardized infant stress paradigm. Fifteen-month-old children of a bipolar parent show a distinctive response to separation compared with control children.

Rapid cycling patients maintained on lithium show a strikingly elevated prevalence of hypothyroidism, both overt and subtle, compared with non-rapid cycling patients on lithium. This finding lends further support to the observation that functioning of the hypothalamic-pituitary-thyroid axis is abnormal in rapid cyclers.

A program of brief outpatient studies of depressive syndromes and character disorders has begun under Dr. Cowdry's direction. Two factors have stimulated our interest in a range of illnesses commonly seen in outpatient community settings. First is the dramatically decreased need for hospital treatment for most major depressions; and second is our interest in exploring the specificity of biological findings by examining a wider spectrum of patients. The bulk of the current expansion of the Outpatient Clinic will focus on these brief studies involving relatively large numbers of patients. Beginning in July, 1980, the Clinic will be staffed by two psychiatrists, full-time, a social worker, half-time, and a nurse expert. We will also begin a training program which will include a psychiatric resident, half-time, and two social work students.

In addition to research in endogenous and atypical depressions, a project investigating possible limbic system dysfunction in the borderline syndrome is under way. In one study reported this year by Dr. Cowdry, a high incidence of symptoms suggestive of limbic dysfunction together with a high incidence of temporal and occipital EEG abnormalities was found in borderline patients compared with patients with unipolar depression.

Basic and clinical studies of the neuronal and glial enolases have continued this year under Dr. Paul Marangos' direction. Immunohistochemical studies have established that one form of enolase is strictly localized in neurons and neuroendocrine cells and it has been named Neuron Specific Enolase or NSE. Another enolase isoenzyme has also been isolated and shown to be localized in glial cells and designated Non-Neuronal Enolase or NNE.

Our research efforts that concern neuronal and glial enolases have focused on the use of NNE and NSE as probes of CNS morphology, pathology, and development and as markers for the diffuse neuroendocrine nervous system.

Important findings this past year include:

A phylogenetic study has shown that NSE is present only in vertebrate nervous tissue, and not in lower species.

The recently demonstrated "switch" from NNE to NSE during neuronal differentiation occurs in cell culture systems as well as in vivo but is less complete.

The use of NNE and NSE staining as an index of neuronal differentiation has been applied to a variety of other neuronal systems, such as the retina and the external granular cell layer of cerebellum. Such studies may enable a correlation between neuronal differentiation indexed by NSE and other specific neuronal events occurring during neuronal development. For example, the switch to NSE is coincident with synaptogenesis in the chick auditory system. Similarly, the method is being applied to the process of dedifferentiation occurring in cancer.

Studies of the anatomical distribution of NSE find that NSE occurs in peripheral neuroendocrine cells of the APUD series, providing evidence for their paraneuronal nature and a means of mapping their distribution.

NSE occurs in platelets, a tissue used as a clinically available model for some aspect of nerve ending function.

NSE occurs transiently during the development of ectodermally derived muscle cells (but not mesodermally derived muscle cells), providing evidence of a specific biochemical similarity between neurons and muscle cells.

NSE is elevated in the amniotic fluid when neural tube defects are present in the fetus, suggesting a clinical application for this assay.

NSE levels are not elevated in schizophrenia. Studies in a variety of neurological disorders are under way.

The demonstration of pharmacologically relevant receptor sites for the benzodiazepines in brain suggests the existence of naturally-occurring endogenous ligands for the brain benzodiazepine receptor, that might provide insight into endogenous mechanisms regulating anxiety, muscular tone, and seizures. Studies from our laboratory have shown that the purines specifically inhibit diazepam binding to brain receptor sites, that they possess benzodiazepine-like pharmacologic and neurophysiologic properties, and that structure activity relationships exist.

Further studies during the past year include the following:

The methylated purines caffeine and theophyllin are potent inhibitors of diazepam binding. Caffeine-induced seizures are antagonized by benzodiazepines and partially antagonized by active purines, suggesting that caffeine and either the benzodiazepines or the active purines form agonist/antagonist pairs for the diazepam receptor.

Diazepam in low doses increases exploratory activity in an animal paradigm developed in our lab by Dr. Crawley. Inosine blocks this activation. This could be interpreted either as antagonism of diazepam effects, or as potentiation leading to sedation.

Preliminary studies suggest that purines are present in vivo in concentrations sufficient to influence the benzodiazepine receptor. Regional distribution studies of brain purines are under way.

It has been well documented that the benzodiazepine and GABA receptor systems are functionally coupled. Both benzodiazepines and purines inhibit GABA-stimulated diazepam binding in relatively low concentrations, suggesting that purines may regulate this coupled receptor system.

The relative potencies of benzodiazepines in inhibiting GABA-stimulated diazepam binding differ from the potencies in inhibiting basal diazepam binding, suggesting either that GABA induces a change in the diazepam receptor or that two populations of diazepam receptors exist.

Treatment of animals with clonazepam for three weeks induces a sub-sensitivity of the diazepam receptor, that persists for about one week following drug discontinuation.

Two additional, higher molecular weight inhibitors of diazepam binding have been found, but not yet identified.

Studies of CNS neurotransmitter and drug receptors are directed by Dr. Steven Paul. Studies of the benzodiazepine receptor complement Dr. Marangos' work with endogenous ligands. In addition, specific high affinity binding sites for imipramine have been identified in brain. Major findings regarding these receptor sites include:

Benzodiazepine-receptor sites similar to those in mammalian brain are found in primary cultures of fetal mouse brain, providing a possible in vitro model for studies of the benzodiazepine-GABA-chloride ionophore complex.

Mammalian retina contains both "brain-type" and "kidney-type" benzodiazepine receptors, providing a model for looking at interrelationships between these receptor populations.

Heterogenous populations of benzodiazepine receptors exist in brain.

Introduction of a bridged compound in the N⁻⁶ position of adenosine results in a compound that increases the number of diazepam receptor sites, possibly through displacement of an endogenous inhibitor of binding. Such compounds seem to possess long-lasting anticonvulsant, anxiolytic, and sedative properties.

High affinity and saturable binding sites for imipramine have been demonstrated in rat brain, human brain, and human platelet. Tertiary amine antidepressants are more potent inhibitors of binding than are secondary amine antidepressants, suggesting a relationship between the binding sites and the serotonin reuptake site.

Imipramine binding sites in platelet are very similar to those in brain, suggesting their use as a peripheral measure of changes in central nervous system receptors in clinical studies.

In preliminary studies platelets from severely depressed patients show a significant decrease (>30%) in imipramine binding site density compared with age-matched controls.

Studies on the interaction of peripheral hormones with CNS neurotransmitter receptors have continued under Dr. Paul's direction. Significant behavioral and neuroendocrine effects of a variety of peripheral hormones including the gonadal steroids, as well as the pituitary and gastrointestinal peptides have been described by a number of investigators. Little data exists as to the neurochemical mechanisms involved in the interaction of these hormones with excitable tissues; including central neurotransmitter systems. Studies in our laboratory have been designed to elucidate the possible biochemical effects of hormones on neuronal preparations in vivo and in vitro.

Biologically active estrogens increase cyclic AMP accumulation in and norepinephrine efflux from hypothalamus, events which may relate to effects on behavior and mood.

High-affinity, saturable binding sites for biologically active ^{125}I cholecystokinin were demonstrated in rat brain membranes, suggesting that this peptide may have CNS effects which are mediated through specific CNS receptor sites.

SUMMARY

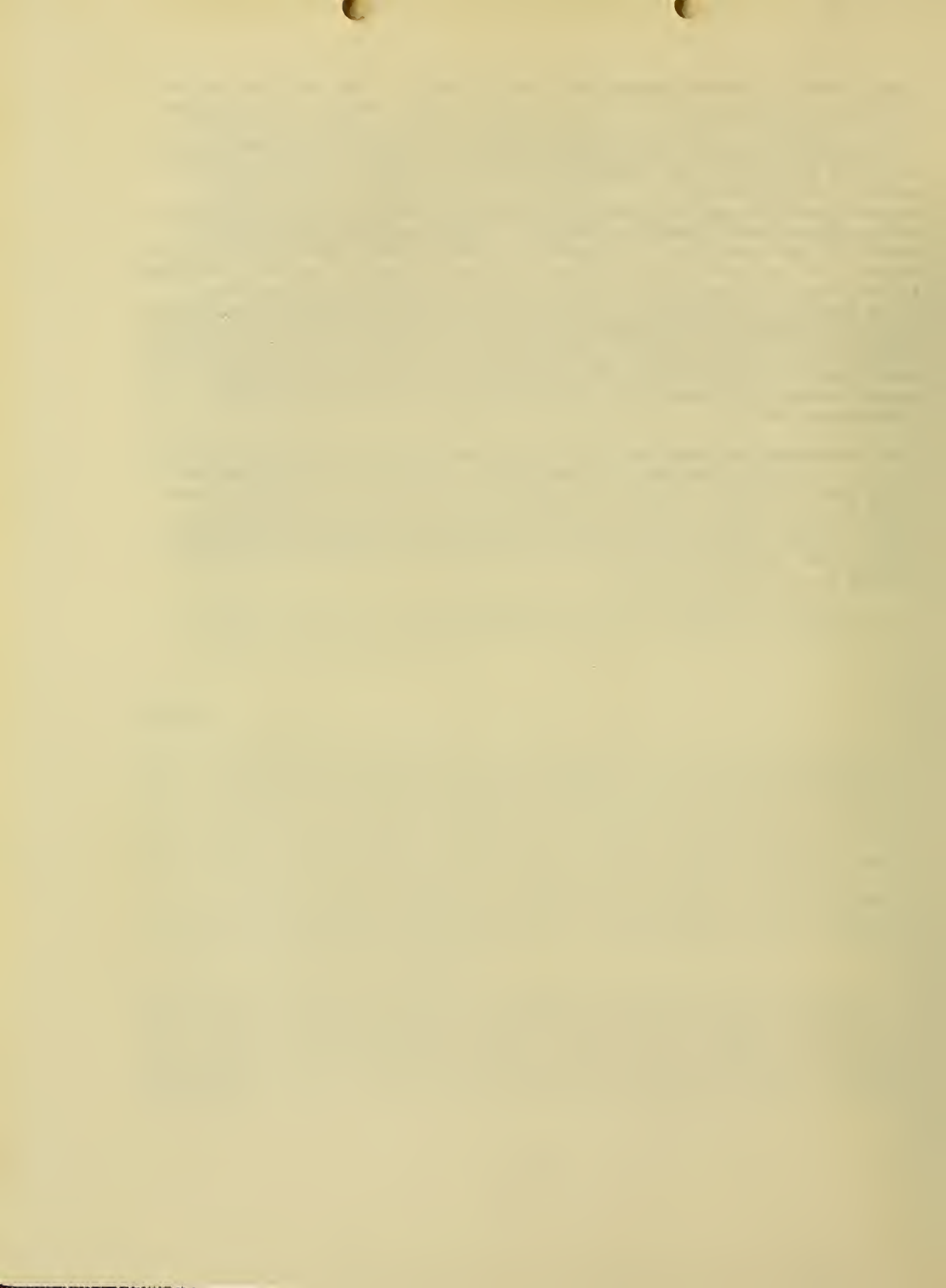
The Clinical Psychobiology Branch has developed the capability to pursue a broad spectrum of interrelated research projects in the major affective disorders. Increases in professional personnel have come largely as a result of our success in recruiting people with "outside" funding such as our three NIGMS PRAT Fellows, and three scientists on foreign fellowships. Of the 19 professionals in the Branch, only 9 require NIMH positions. Two major gaps in our research efforts are apparent: the relative absence of neurophysiological research and the absence of psychological research. The first gap in expertise was addressed this year when Dr. Richard Ross and Dr. Jacqueline Crawley joined the Branch as PRAT Fellows. The absence of a psychologist in the Branch remains to be remedied.

Laboratory research will continue to emphasize projects which complement the clinical research enterprise: clinical assay development; research on drug effects on neurotransmitter systems and circadian rhythms; identification of drug and neuroendocrine receptor sites and their endogenous ligands; development of animal behavior models; and the exploration of basic neuronal biochemistry.

Space remains a chronic problem in a growing branch. The completion of the Ambulatory Care Research Facility will significantly relieve this pressure.

Finally, the success of this research enterprise requires the active participation of a variety of patients with affective disorder. The NIH operates entirely through referrals. Increasingly, practitioners have referred primarily patients with refractory or otherwise unusual clinical conditions, while patients with "typical" affective illness have been satisfactorily treated in the community. In an effort to address this problem of selective referrals to our relatively long-term inpatient protocols, we have emphasized the development of an active outpatient unit, providing consultation and brief research involvement for a broader and more representative spectrum of depressed patients. Since these "outpatient" programs involve only brief inpatient stays for evaluation, they have greater appeal both for the potential research subject and for the referring physician, who can continue psychotherapy with the patient throughout the patient's evaluation and treatment at NIH.

Thus, we expect the inpatient unit to continue its extensive longitudinal studies of rapid cycling bipolar patients to elucidate specific relationships among circadian rhythms, neuroendocrine systems, and monoamine neurotransmitters. At the same time, the unit will provide an intermediate length-of-stay capability for studies such as the electroconvulsive therapy protocol and a brief length-of-stay capability for patients evaluated and treated by the outpatient unit.



Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health

Irwin J. Kopin, M.D., Chief

October 1, 1979 through September 30, 1980

Introduction

During the last year the research programs of the Laboratory of Clinical Science have continued to be directed at elucidating the fundamental processes which are responsible for the development and function of the nervous system, at developing methods and approaches to evaluation of these processes in intact animals and humans, and at applying these concepts and methodological advances to clinical investigations. The goals are to determine the biological bases of disordered brain function and to provide rational approaches to chemotherapy of neurological and psychiatric disorders. The research programs are carried out by four Sections (Pharmacology, Histopharmacology, Medicine, and Experimental Therapeutics) and three Units (Clinical Biochemistry, Pharmacological Applications of Mass Spectrometry, and Neuroendocrinology). The Units are in the Office of the Chief and in addition to their own research programs, provide laboratory support for clinical investigations.

The Laboratory is organized and directed in a manner which aims to provide ample opportunity for interactions among the basic scientists and clinical investigators. This encourages rapid application of newly developed concepts or methods to clinical problems and takes advantage of clinical problems in stimulating new direction in fundamental research. Free interaction, wide-spread collaboration, and opportunity to explore new ideas provide an environment in which creativity, resourcefulness, and dedication of scientists can best flourish. Thus, although the various sections in the Laboratory each have independent research programs, the common interests in fundamental processes result in a network of collaborative efforts among the sections, other branches of the NIMH and other institutes.

Section on Pharmacology Julius Axelrod, Ph.D., Chief,

The main effort of the Section on Pharmacology has been directed toward the role of lipids in the transduction of biological signals through membranes. Stimulation of IgE receptor or mast cells, or basophilic leukemic cells by suitable antigens causes a transient increase in phospholipid methylation, Ca^{2+} influx, phospholipase A_2 activation (as indicated by release of arachidonic acid) and histamine release. Blocking phospholipid methylation inhibited Ca^{2+} influx, arachidonic acid and histamine release. A close relationship between phospholipid

methylation Ca^{2+} influx, phospholipase A_2 activation and biological response was also found in rabbit leucocytes, human fibroblasts and mouse lymphocytes. Phospholipids were found to be regulated by a specific inhibitory protein that is induced by corticoids (Drs. Hirata, Crews, and Axelrod).

In a related study it was observed that in rats dependent on ethanol there is a decrease in the activity of phospholipid methylating enzyme in synaptosomal plasma membranes. There is also an increase in membrane viscosity and cholesterol content in synaptosomal membranes of alcohol-dependent rats.

Calmodulin, a protein that regulates the activity of a number of Ca^{2+} -requiring enzymes in nervous and other tissues, is an excellent substrate for protein carboxymethylase. When calmodulin is methylated its activity is reduced. The acetylcholine receptor, a complex of 5 polypeptides, is also a good substrate of protein carboxymethylase.

Several tissues (lung, retina, brain) incorporate radioactive methyl groups from S-adenosyl methionine into lipids by an enzymatic process. One group of methylated lipid has been found to be fatty acid esters. The remaining lipids are not yet fully identified (Drs. Zatz and Markey).

The role of catecholamines in the central and peripheral nervous systems in experimental and genetic hypertension and stress was extended to a study of the participation of other biogenic amines (serotonin, histamine) and neuropeptides. Alterations of β -adrenergic receptors and endogenous opiate peptides were found in genetic hypertensive rats. Changes in the metabolism of membrane phospholipids were also found in these rats. In addition to catecholamines, serotonin and histamine are involved in the regulation of sodium intake and in the formation of hypothalamic vasopressin (Drs. Saavedra and Correa).

The delay in the onset of action of antidepressant therapy is a major clinical problem. Studies in rats have shown that desensitization of cerebral cortical β -adrenergic receptors requires prolonged treatment with antidepressant drugs similar to the time course required for the onset of therapeutic actions. Combined treatment with the antidepressant desipramine and the α -adrenergic blocking agent phenoxybenzamine causes rapid desensitization of cerebral cortical β -adrenergic receptors (Dr. Crews).

Catechol estrogens are major metabolites of estrogens and have important biological properties of their own. They were found to be synthesized in the brain and liver by 2-hydroxylation of estrogens. They can also be formed by the demethylation of 2-methoxyestrogens in the liver. Both the brain and the liver estrogen-2-hydroxylase can be measured in fetuses and show

sexual dimorphism during development. Administration of testosterone just after birth decreases estrogen-2-hydroxylase. Given later on this steroid increases enzyme activity (Dr. Hoffman).

Section on Histopharmacology
David M. Jacobowitz, Ph.D., Chief

This section has been involved with studies of mono-aminergic and cholinergic neurons in the central nervous system. More recently the immunocytochemical and neurochemical evaluation of peptide-containing nerves in the brain and periphery has been pursued. Histochemical and biochemical analyses are carried out at the level of discrete central nuclei and pathways in order to define the underlying basis for behavioral changes.

The chemistry, physiology and pharmacology of the α -melanocyte stimulating hormone (α -MSH) system was studied. By combining high pressure liquid chromatography with a radio-immunoassay, α -MSH and deacetylated α -MSH and their respective methionine sulfoxide forms were identified. By studying the effects of these peptides on behavior, it was determined that deacetylated α -MSH is an inactive form and probably a precursor to α -MSH. α -MSH is released in vitro by veratridine or potassium stimulation in a calcium dependent manner. Stimulation of the central gray matter causes in vivo release of α -MSH into the cerebrospinal fluid. Acute, but not chronic stress, also causes a dramatic decrease in brain α -MSH concentrations which is interpreted to indicate a release of α -MSH. A diurnal rhythm of α -MSH in the brain was shifted by clorgyline treatment. α -MSH concentrations in the brain were decreased by fluphenazine treatment and increased by housing rats in continuous darkness (Mr. O'Donohue, Drs. Helke, Torda, Saavedra, Thoa, Weir, Kafka, and Wirz-Justice).

Evidence that substance P (SP) is a neurotransmitter of baro- and/or chemoreceptor afferent nerves has been obtained by (1) measurement of the immunoreactive SP (SP-I) content of the nucleus tractus solitarius (NTS) in the rat following unilateral nodose ganglionectomy; (2) utilization of immunocytochemistry to detect the presence of SP in peripheral baro- and chemoreceptive regions and pathways of the afferent fibers; and (3) investigation of the potassium-stimulated release of SP from the NTS in vitro. It was found that the NTS contained significant amounts of SP-I and that unilateral removal of the nodose ganglia reduces the SP-I content of those portions of the NTS known to receive vagal afferents. In addition, SP-I was visualized in discrete fibers in the tunica adventitia of the aortic arch and carotid sinus regions, the carotid body, the vagus nerve and nodose ganglia. Stimulation of freshly dissected slices of the NTS region with 50 mM potassium in the presence of calcium

increases SP release 2-3 fold. Omission of calcium from the buffer abolished completely SP release by K^+ . These results provide evidence that SP is contained within baro- and chemoreceptor afferent nerves (Dr. Helke, Mr. O'Donohue, and Dr. Thoa).

Choline acetyltransferase (ChAT) activity was measured in numerous discrete brain nuclei of DOCA-salt hypertensive rats. Three control groups were employed. These rats received either (1) normal drinking water, no DOCA, (2) saline (1%) drinking water, no DOCA, (3) normal drinking water plus DOCA. Changes in the ChAT activity of the dorsomedial nucleus of the hypothalamus and the intermediate portion of the nucleus tractus solitarius were found in the DOCA salt hypertensive rats compared to the normotensive control rats. In addition, a decline in the ChAT activity of the supraoptic nucleus was noted in the 2 groups of salt-treated rats. The former changes are likely related to the elevated blood pressure while the latter appear to be a response to chronic salt treatment rather than elevated blood pressure (Drs. Helke and Sohl).

A retrograde flow mapping technique that is specific for dopamine- β -hydroxylase (DBH) containing neurons was used to trace the cell body origins of noradrenergic neuronal projects to the cerebellum. Following unilateral or midline injection of the antibody to DBH (ADBH), noradrenergic cell bodies were observed to contain the ADBH in the pontine tegmentum. This work demonstrates that in addition to the well known locus coeruleus project to the cerebellum, projections from the sub-coeruleus, A5 and A7 groups of cells exist. All cell groups projected both ipsilaterally and contralaterally (Dr. Pasquier and Mr. Gold).

In rat brain changes in choline acetyltransferase activity following stereotaxic lesions revealed that cholinergic nerves of the nucleus of the tractus diagonalis project to the cortex (frontal, cingulate, entorhinal, occipital), hippocampus, amygdala, dorsomedial nucleus, lateral habenula, globus pallidus, anterior ventral nucleus of the thalamus and interpeduncular nucleus. The nucleus tractus diagonalis is a major cholinergic nucleus that projects long descending neurons to regions of the forebrain, midbrain and hindbrain (Dr. Helke, Mr. Muth, and Mr. Gold).

Previous work on this project established a feedback effect of gonadal hormones on the cholinergic activity of the n. tractus diagonalis (td) and the ventral tegmental area (avt) of the rat brain, concomitant with hormone-induced alterations in luteinizing hormone (LH) secretion. The present work suggests that (a) the changes in cholinergic activity are secondary to the alterations in LH secretion, rather than causative; (b) the changes may in fact result from gonadal hormone-induced alterations in pituitary

trophic hormone secretion since hypophysectomy prevents them; (c) the observed changes in cholinergic activity are a physiological manifestation of a neuroanatomical link between the mesolimbic dopamine (DA) system originating in the avt and the cholinergic system originating in the td (Mr. Muth).

A discrete regional analysis of norepinephrine, dopamine, choline acetyltransferase and glutamic acid decarboxylase was performed in the brain of the newborn and pubescent monkey. A method was described for the removal of discrete areas of the monkey brain. Comparison between newborn and pubescent monkeys showed a 3-4.5-fold increase in DA in the caudate and putamen of the pubescent monkeys and a 1.5-4.0 increase in CAT activity in most areas. The GAD activity was generally lower in the newborn when compared to the pubescent brain except for the sensory cortex and parts of the basal ganglia which showed a significant increase (Drs. Speciale, Gottesfeld, Kato, O'Neill, Caveness).

Section on Experimental Therapeutics
Michael H. Ebert, M.D., Chief

The Section on Experimental Therapeutics has continued to study a variety of neuropsychiatric disorders, including Alzheimer's dementia, Gilles de la Tourette's syndrome, hyperactive syndrome of childhood, Anorexia Nervosa, and chronic orthostatic hypotension resulting from autonomic nervous system dysfunction or other causes. Studies of catecholamine metabolism in vivo in primates and in man have complemented these studies.

Substantial progress has been made on the development of a method to estimate the rate of brain homovanillic acid (HVA) production by a relatively non-invasive technique utilizing blood samples only to examine the kinetics of HVA distribution and its elimination before and after administration of probenecid. Studies utilizing this technique have been completed in Rhesus monkey (Drs. Elchisak, Ebert, and Kopin). In these animals the basal rate of HVA production by the whole body, including brain, was 1.0-1.4 mg/day. The amount of HVA derived from the brain was estimated to be 55-63 percent of the total daily production.

Preliminary studies on brain HVA production have been similarly conducted in three human normal volunteers (Drs. Elchisak, Polinsky, Ebert, and Kopin). The decline of plasma d₅-HVA concentration with time is biexponential, with a biological half-life ranging from 63 to 79 minutes. During probenecid infusion, the decline of plasma d₅-HVA was triexponential in two patients, and biexponential in the third. The biological half-life was 113-170 minutes. The calculated basal rate of HVA production was 6.2-7.8 mg/day. The amount of HVA derived from the brain in these volunteers was calculated to be 51-75 percent of the total daily production.

Dopamine sulfate (DASO₄) is a metabolite of dopamine (DA) which occurs in several animal species. Work has continued to demonstrate the occurrence and possible physiological significance of this compound as a metabolic intermediate in primates, including humans (Drs. Elchisak and Ebert). Conjugated DA, primarily DASO₄, occurs in much greater concentrations than free DA in all body fluids studied thus far. Excretion of free DA in human urine is approximately 2-4 μ moles/day, while the excretion of conjugated DA, primarily as DASO₄, is 4-8 μ moles/day. The concentration of free DA is approximately 0.5-1 picomole/ml in human serum, whereas, conjugated DA is 20-30 picomoles/ml. Both DA-3-SO₄ and DA-4-SO₄ isomers have been detected in urine from Rhesus monkeys, but only DA-3-SO₄ has been found in human urine.

Levels of free DA in CSF taken from the lateral ventricle of the Rhesus monkey are approximately 0.75 picomole/ml and conjugated DA is 2-3 picomole/ml. In lumbar spinal CSF, free DA is approximately 0.25 picomole/ml, and conjugated DA is about 3 picomoles/ml. Preliminary results from intravenous infusion of ¹⁴C-DASO₄ into Rhesus monkeys suggest that neither ¹⁴C-DA-3-SO₄ nor ¹⁴C-DA-4-SO₄ crosses the blood-brain or blood-CSF barrier in this species. This suggests that the substantial quantities of DASO₄ found in the CSF come from the brain or spinal cord, and not from the blood. These same experiments showed that steady-state levels of total radioactivity were approached within four hours after beginning the constant-rate infusion. The biological half-life of ¹⁴C-DASO₄ is, therefore, one hour or less in the Rhesus monkey.

Two new clinical studies of catecholamine metabolism have begun this year. A technique to study epinephrine turnover in man is being developed by using deuterated methionine to label the N-methyl group of epinephrine during its synthesis in the adrenal gland. The technique will be used to study the clinical pharmacology of SKF 64139, a PNMT inhibitor. In collaboration with Dr. Robert Kessler (Nuclear Medicine Department, Clinical Center), preliminary studies are in progress to develop a labeling technique for brain dopamine neurons that would be applicable to position emission tomography. This effort involves the production of ¹⁸F labelled precursors of dopamine or dopamine receptor ligands whose pharmacology is not different from the non-labelled species.

Neuropharmacological assessment of autonomic dysfunction has continued in patients with chronic autonomic failure (Drs. Polinsky, Kopin, Weise, Ebert). Analysis of the glucose recovery curves following insulin-induced hypoglycemia in patients with orthostatic hypotension shows that there is a mild insulin resistance in those patients with absent adrenal medullary responses. Basal cortisol levels are elevated in patients with

adrenergic insufficiency; the sequence, magnitude, and temporal profile of glucagon, growth hormone and cortisol are unaltered despite the absent catecholamine response to hypoglycemia in these patients. During insulin-induced hypoglycemia, pancreatic levels in plasma normally reach a maximum at 50 minutes after administration of insulin. In patients with idiopathic orthostatic hypotension there is a deficiency in plasma pancreatic peptide levels response; a patient with multiple system atrophy showed variable pancreatic peptide responses. Another hormone which has been measured in response to hypoglycemia is gastrin. Patients with idiopathic orthostatic hypotension have elevated fasting levels of gastrin with a normal increment following hypoglycemia. Multiple system atrophy patients have normal fasting gastrin levels but a reduced increment in response to hypoglycemia.

Examination of CSF levels of norepinephrine has shown that those patients with Idiopathic Orthostatic Hypotension have low CSF norepinephrine compared to controls; the CSF level in patients with Multiple System Atrophy is either normal or elevated. The neural control of melatonin rhythm is exerted through the beta adrenergic receptors. Measurement of 6-hydroxymelatonin in the urine (in collaboration with Drs. Tetsuo and Markey, Mass Spectrometry Unit) reveals that some patients with orthostatic hypotension have very low levels of melatonin while other patients have a normal amount but altered diurnal pattern of melatonin secretion.

Measurement of ligand binding to alpha receptors of red blood cells and platelets is thought to be parallel changes which occur at the vascular alpha receptor. Preliminary analysis of this data by Dr. Marian Kafka, BPB, suggests that male Shy-Drager syndrome patients and female Idiopathic Orthostatic Hypotension patients have increased alpha receptor sites.

Clinical evaluation of orthostatic hypotension patients has shown that the blood volume and RBC mass, as measured by Cr^{51} -RBC labelling, is reduced in about two-thirds of the patients. Assay of serum 2,3-DPG and erythropoietin levels are in progress as well as an attempt to correlate the anemia with changes in renal function. This work is done in collaboration with Dr. Laurence Corash, Hematology Service.

In Gilles de la Tourette's syndrome (TS), a therapeutic trial of lecithin has been completed. Although there were some minor improvements in either motor or vocal tics in individuals, the overall group responses showed no difference compared to the placebo treatment. Neurological examination of 48 patients with TS revealed that 57% exhibit "soft neurologic signs." Haloperidol remains the treatment of choice for all patients requiring medication in our outpatient clinical population.

Pharmacological studies of memory function in Alzheimer's disease have continued this year in collaboration with Drs. Weingartner, Sitaram and Gillin. Various parameters of memory processing and retrieval are being documented by Dr. Weingartner. Several pharmacological approaches to modifying brain cholinergic function have been studied. Infusion of arecholine (a cholinergic receptor agonist) produces transient improvement in serial learning tasks. A trial of lecithin (a dietary source of choline) and THA (an oral cholinesterase inhibitor) used in combination produced a significant improvement in memory function in five of nine patients studied.

A clinical genetic study of familial Alzheimer's disease is nearing completion (Ms. Nee, Drs. Polinsky, and Ebert). A single family has been studied, and the pedigree has the following characteristics: 9 generations, 821 identified individuals, 34 individuals with a presumptive diagnosis of Alzheimer's disease, 2 individuals with a neuropathological diagnosis, 9 individuals living with a clinical diagnosis, 54 individuals evaluated at the NIMH, 111 individuals from whom blood samples were obtained. A formal genetic analysis of HLA antigens is in progress in collaboration with Dr. Weitkamp, University of Rochester.

The clinical research program in Primary Anorexia Nervosa has continued (Drs. Kaye, Ebert, and Lake). A study of central nervous system dopamine, serotonin, and norepinephrine metabolism was completed using CSF levels of amines and amine metabolites. Four groups were compared: Anorexia Nervosa patients at minimal weight, the same patients immediately after weight gain, "recovered" Anorexia patients at normal weight for six months but with continued psychiatric symptoms, and normal female controls. At minimal weight a 40% decrease in HVA and a 20% decrease in 5HIAA was observed compared to the other three groups which did not differ from each other. This indicates a decrease in dopamine and serotonin turnover in the acutely ill patient with Anorexia Nervosa. In contrast, CSF norepinephrine levels are significantly lower in the group of "recovered" Anorexia patients compared to the other groups. In collaboration with Dr. Gold (CPB, NIMH) and Dr. Robertson (University of Chicago), vasopressin (AVP) function was studied. Anorexia Nervosa patients have an erratic secretory response of AVP to a hypertonic saline infusion compared to the linear change in AVP to increasing plasma osmolality in normals. Anorexia Nervosa patients have a high CSF/plasma ratio of AVP compared to normals. This CSF AVP abnormality is most extreme in the "recovered" group above, and CSF AVP levels have a reciprocal relationship to CSF NE levels. These neurochemical data provide additional evidence of an abnormality in hypothalamic function in these patients, and suggest that the abnormality may persist after immediate weight gain. Norepinephrine and AVP have both been implicated in the regulatory mechanisms of appetite in animals.

A series of pharmacokinetic and metabolic studies of phencyclidine have been carried out in animals (Drs. Burns, Ebert, and Hawks). Pilot studies in rats demonstrated that the urinary excretion of phencyclidine is pH-dependent with a two- to three-fold increase on acidification. However, the relationship between the pKa, the permeability of the unionized moiety, and the metabolic rate is such that no significant change in the net quantity of drug excreted is observed. Using a previously described gas chromatography-mass spectrometry assay for phencyclidine, the kinetics of the drug, and the relationship between the neurological effects and the plasma levels were studied in the monkey. The kinetics are well described by a two-compartment model with an elimination half-life of 3.5 hours. The termination of phencyclidine-induced nystagmus is related to plasma levels of phencyclidine in the range of 20 to 30 ng/ml. Pharmacokinetic studies of phencyclidine in man are planned during the next year.

Collaborative projects continue with the Childhood Mental Illness Unit of the BPB, NIMH on the clinical pharmacology of stimulants, L-dopa, and tricyclic antidepressants in the treatment of hyperactive children. Collaborative research continues with Dr. Merrill, LGCB, NIMH on clinical applications of two-dimensional polyacrilamide gel electrophoresis of proteins, particularly in the study of central nervous system genetic disease.

Section on Medicine
Irwin J. Kopin, M.D., Chief

The central nervous system mediates compensatory physiological responses to environmental stimuli, stress, and alterations in body temperature, O₂ and CO₂ content of blood, blood glucose levels, etc. The magnitude of the responses are influenced by the intensity of the afferent stimulation which reaches the central nervous system (CNS), the responsivity of the appropriate nuclei in the CNS, and the sensitivity and capacity of the effector organ to respond to the neurohumors released from peripheral nerve endings. Plasma levels of catecholamines and the alterations in those levels which normally attend postural change, physical exertion, or stress are valid indices of sympatho-adrenal medullary activity in patients as well as in experimental animals. The responses to administered pressor agents, particularly in animals in which the compensatory reflexes have been prevented, either pharmacologically or by destruction of the central nervous system, allows assessment of effector organ responsivities separately from neuronal influences.

In pithed rats stimulation of the sympathetic outflow from the spinal cord elevates plasma catecholamine levels and

elevates blood pressure. By blocking presynaptic α_2 -type adrenoceptors modulation of norepinephrine release, α -adrenoceptor blocking agents enhance the stimulation-induced increase in plasma norepinephrine levels and by blocking α_1 -type receptors on smooth muscle of the blood vessels these agents prevent the rise in blood pressure. Some α -adrenoceptor blocking agents affect preferentially one type of α -adrenoceptor. By correlating the potencies of a series of α -adrenoceptors on the sympathetic stimulation-induced increase in blood pressure and the use in plasma catecholamines with the effects on the pressor response to administered catecholamines, it has been found that an α_2 -type adrenoceptor mediates the response to exogenous norepinephrine. This indicates that there the two types of α -adrenoceptors are present on vascular smooth muscle - α_1 -type at junctions of sympathetic nerves with the muscle and α_2 -type at extrajunctional sites (Drs. Yamaguchi and Kopin).

Pithed spontaneously hypertensive (SHR) rats have greater pressor responses to sympathetic stimulation than do normotensive (WKY) rats of the same strain, but similar increases in plasma catecholamines and at a young age similar responses to administered norepinephrine. The SHR rats appear to have deficient β -adrenoceptor vasodilatory responses as well as enhanced α_1 -type adrenoceptor sensitivity (Drs. Yamaguchi and Kopin). Rats with experimental renal or DOCA-salt hypertension also have enhanced pressor responses to sympathetic stimulation although normal or slightly diminished amounts of norepinephrine are released.

Hemorrhage elicits reflex sympatho-adrenal medullary activation and increases plasma catecholamine levels in anesthetized rats (Drs. Feuerstein and Kopin). Prostaglandins of the E-series elevate blood pressure and heart rate, enhance the sympathetic response, and increase the survival of hemorrhaged rats. These effects appear to be mediated by a renal factor, since they are not seen in nephrectomized hemorrhaged rats.

Prostaglandins of the $F_{2\alpha}$ series also elevate blood pressure and heart rate, but act through the central nervous system. Specific forebrain nuclei which mediate these responses have been identified and are being explored (Drs. Feuerstein, Jacobowitz, and Kopin).

Dopaminergic neurones in brain appear to be sensitive to changes in blood glucose levels, decreasing their firing rate and releasing less dopamine (as indicated by a decrease in dopamine metabolites) when blood glucose is elevated (Dr. Saller). Even smaller amounts of glucose, comparable to the amounts consumed during a meal or released by the liver during stress, suppress both the firing of dopaminergic neurons in the brain and central dopamine release. In addition, glucose administration can modify the response of central dopaminergic neurons to

the antipsychotic agent, haloperidol. Haloperidol administration normally produces a large increase in impulse flow along dopaminergic axons and activates tyrosine hydroxylase, thought to be the rate-limiting enzyme in catecholamine synthesis. However, glucose administration can either prevent or suppress the increased firing of dopamine-containing neurons normally elicited by haloperidol, but it does not prevent tyrosine hydroxylase activation. The latter finding suggests that haloperidol and perhaps other antipsychotic drugs may influence central dopamine metabolism independently of their effects on impulse flow. A simple yet sensitive and specific assay for the analysis of O-methylated catecholamines in both tissues and urine has been developed and is being applied to measure central and peripheral catecholaminergic activity in animals and human subjects (Drs. Saller, Oddershede, and Lozovsky). Conjugates of O-methylated amines have been found in rat brain.

In rats, 3-methoxy-4-hydroxy-phenylglycol (MHPG) is the major urinary metabolite of epinephrine and norepinephrine. It is excreted mainly as sulfate or glucuronide conjugates, but a small portion of MHPG is further metabolized to VMA (Dr. Lozovsky). In humans, MHPG is largely metabolized to VMA, but in rats, even with blockade of sulfate and glucuronide formation, MHPG is not converted to MHPG to the extent seen in humans. This suggests that rats have relatively low levels of the enzymes responsible for oxidation of glycols.

In humans as well as in experimental animals plasma norepinephrine levels reflect sympathetic nerve activity. In most patients with hypertension plasma catecholamine levels are normal, but in a subgroup of young persons with high blood pressure, plasma norepinephrine levels are elevated. Administration of diuretics lowers blood pressure in hypertensives; the hypotensive effect appears to elicit a sympathetic response which can sometimes compensate for the tendency of the blood pressure to fall and interfere with the therapeutic effects of the diuretics. Fenfluramine has been shown to diminish this reflex-mediated increase in sympathetic activity and thereby enhance the therapeutic effects of hydrochlorthiazide (Drs. Lake, Ziegler, Coleman, and Kopin).

Using direct ligand binding to adrenergic receptors in homogenates of brain, heart, lung, and spleen, forced immobilization stress or repeated administration of isoproterenol have been shown to diminish the number of β_1 -type and to alter adrenoceptors. Changes in receptor density appear to be prevented by administration of quinacrine, a phospholipase A_2 inhibitor. The physiological effects of catecholamines on heart rate and blood pressure parallel the changes in receptor density (Drs. Torda, Yamaguchi, Hirata, Kopin, and Axelrod).

Intravenously administered ^3H -dihydroalprenolol can be used to label β -adrenoceptors in vivo and its displacement with stereospecific β -adrenoceptor Blocking agents can be used to develop a method to assess β -receptor density in humans (Drs. DiMicco, Kopin and Pert).

During development of the cerebellum of rats, enzymes which control synthesis of neurotransmitters appear and increase in concentration at different times, while enzymes which control polyamine synthesis increase only transiently and apparently are involved in triggering different phases of development (Drs. Gilad and Kopin). After axonal injury, changes in enzyme levels result in a pattern similar to that seen during development.

A radioactively labelled irreversible inhibitor of glutamic acid decarboxylase (GAD) was successfully used to localize the enzyme after two-dimensional electrophoresis. Antibodies to the enzyme have been produced and have been used to demonstrate the presence of GAD in specific cells in the nervous system (Dr. Oertel and Mrs. Weise). Since this antibody inhibits GAD activity in parallel with cysteine sulfonic acid decarboxylase activity, it is likely that the same enzyme catalyses decarboxylation of both amino acids (Dr. Oertel and Mrs. Weise). A second enzyme which decarboxylates only cysteine sulfonic acid is present in liver and brain. This enzyme has different physical characteristics from GAD and is being purified with the objective of making antibodies with which to localize histologically the distribution of the enzyme in brain.

Office of the Chief
Irwin J. Kopin, M.D., Chief

Of the three units in the Office of the Chief, two have developed independent research programs similar in magnitude and scientific impact to those of a Section of the Laboratory. It is anticipated that the Unit on Neuroendocrinology (Dr. Michael Brownstein) and the Unit on Pharmacological Applications of Mass Spectrometry (Dr. Sanford Markey) will be elevated to the status of Sections during the next year. The Unit on Clinical Biochemistry (Mrs. Edna Gordon), which also carries on independent methodological research, works in close collaboration with the Sections on Medicine and Experimental Therapeutics and the Unit on Pharmacological Applications of Mass Spectrometry and is best continued as a Unit within the Office of the Chief.

The members of the Unit on Neuroendocrinology have continued to study biologically active peptides. The peptides are present in central and peripheral nerve cells: these neurones must be located and characterized. In addition, information is

required about the synthesis, axonal transport, and release of the peptides and about the regulation of these processes. Those objectives are essential to understanding the roles of biologically active peptides in the brain and periphery.

Studies on the biosynthesis of vasopressin, oxytocin, and neurophysin (Np) have continued (Drs. Brownstein, Russell, and Gainer). The vasopressin/Np and oxytocin/Np precursors have been described and a third Np precursor discovered. In the course of this work, there has been isolated a glycopeptide that is likely to constitute part of the N-terminus of the vasopressin/Np precursor and, in collaboration with C. Bennett, its structure is being determined. An antichemotactic factor from tumor cells is in the process of being purified and characterized (Drs. Brownstein and Schiffmann). This factor may enable the tumor to protect itself from attack by the host.

The details of regulation of α -MSH secretion from the intermediate lobe of the pituitary by catecholamines has been described (Drs. Eskay and Kebebian).

A number of neuropeptides have been found in mammalian and amphibian retinæ and, more recently, immunocytochemistry has been used to localize these peptides to specific cells (Dr. Eskay). Along similar lines, methods for dissociating and segregating retinal neurons have been developed (Drs. Schaeffer and Brownstein). Large numbers of photoreceptor and ganglion cells can now be prepared for biochemical studies.

The distribution of cholecystokinin (CCK) in the brain has been described and the immunologically active material in brain extracts characterized using high performance liquid chromatography (Drs. Beinfeld and Meyer). The studies have focused on the hypothalamo-neurohypophyseal system and have shown the CCK is present in the posterior pituitary and released in parallel with oxytocin and vasopressin.

The innervation of the supraoptic nucleus has been examined (Dr. Meyer) and a natriuretic factor partially characterized. In a related study the angiotensin-like material present in gut and brain is being characterized (Drs. Furness and Phillips).

Partial characterization of the LHRH-like peptide in frog sympathetic ganglion has been accomplished (Dr. Eiden). This peptide has received a great deal of attention from electrophysiologists; it is not identical to synthetic LHRH. Dr. Eiden is currently engaged in studying the regulation of enkephalin biosynthesis and release in cultured adrenal chromaffin cells.

The retinal toxicity of methylazoxymethanol (MAM), which produces a fairly unique retinal lesion, is being studied by Drs. Eiden, Latker, and Zatz. In a series of immunocytochemical studies of neuropeptides in the gut, several peptides (e.g., somatostatin, bombesin) have been localized to specific neuronal groups and the immunoreactivity characterized by means of high performance liquid chromatography (Dr. Furness).

The research of the Unit on Pharmacological Applications of Mass Spectrometry has developed along lines which are independent, yet supportive of other research goals of the Laboratory. New methods for synthesis of stable isotope-labelled compounds for use in human clinical research studies has been pursued (Dr. Markey). The norepinephrine metabolite, 3-methoxy-4-hydroxyphenylethylglycol (MHPG) has previously been labelled with deuterium on the methyl group, but only the racemic form of the compound could be synthesized. A method for resolution of VMA and subsequent reduction of the desired enantiomer yielded the physiologic isomer D(-) - CD₃-MHPG. This compound was used to study the metabolic fate and kinetics of MHPG in normal human volunteers. With Dr. Wilfried Mayer and Mr. Oliver, ¹⁸O₂-labelled dopamine was prepared via acid catalyzed exchange with H₂¹⁸O. It had previously been reported that phenolic oxygens were not exchangeable, and consequently a significant effort has been made to better understand the chemistry of the catechol oxygen. Dr. Mayer has developed a high yield general synthetic preparation of ¹⁸O-labelled catechols from 1,2 difluorbenzenes activated toward nucleophilic substitution via their chromium tricarbonyl complexes. Syntheses are in progress to prepare ¹⁸O-labelled catecholamine metabolites via these complexes, particularly for those compounds with a benzylic hydroxyl function which preclude the simple direct exchange which had been successfully employed for dopamine.

Drs. Tetsuo and Markey have continued their studies of urinary 6-hydroxymelatonin, the major metabolite of the pineal hormone melatonin, using a sensitive and specific electron capture negative chemical ionization mass spectrometric assay. Diurnal variation in normal human adult males has demonstrated a clear rhythm in the pattern of excretion which parallels that previously observed for melatonin in plasma - approximately 10 times as much metabolite is formed at night in comparison to daytime. In contrast, patients with peripheral or central noradrenergic deficits (orthostatic hypotension) exhibit markedly altered or abolished rhythms, consistent with sympathetic innervation of the human pineal. In collaboration with Dr. Polinsky, Dr. Tetsuo has determined that patients with peripheral deficits have very low melatonin production and no day-night variation; those with central nerve system involvement also exhibit constant melatonin production but of varying basal rates. Dr. Tetsuo and Poth have initiated a study of melatonin production with respect to pubertal stage in children. Drs. Tetsuo and

Perlow have observed the suppression of melatonin production in primates by light, and are examining the effect of pinealectomy on metabolite production. Drs. Tetsuo and Markey have modified one mass spectrometer for high performance capillary gas chromatography using fused silica columns in an effort to extend the dynamic range of the melatonin metabolite assay.

Drs. Lewy and Markey have applied their previously developed mass spectral assay of melatonin to determine the effects of light on normal human melatonin production. Light of intensity comparable to daylight was found to suppress plasma melatonin levels from the normal nighttime rise. When lights were reduced in intensity to normal room levels or turned off, melatonin release rebounded to the normal night levels.

Collaborative projects centered on the identification of methylated lipids (Dr. Zatz, Section on Pharmacology), and liquid chromatographic resolution of optical isomers of β -adrenergic blocking agents (Dr. Kopin) are also in progress.

The Unit on Clinical Biochemistry continued to develop and apply new procedures for the quantitative analysis of biogenic amines and their metabolites in animal and human tissues and biological fluids. Within the Laboratory of Clinical Science, clinical studies have been carried out in collaboration with Drs. Kopin and Ebert. Since these studies increasingly take advantage of the use of stable, as well as radioactive isotopes, the analytical procedures frequently depend on the use of GLC-MS equipment.

Studies attempting to measure metabolites from the central nervous system using ^{14}C dopamine infusions in humans are continuing with Drs. Kopin, Ebert, Polinsky and Kay. The separation of ^{14}C norepinephrine from ^{14}C dopamine metabolites is very difficult and time consuming and occasionally unreliable. Mr. Oliver and Dr. Markey have prepared ^{18}O -dopamine, with this compound, separations and analyses using GLC-MS will be much easier and more dependable.

A study of the rate and extent of conversion of D_3 -MHPG to D_3 -VMA in blood and urine of monkeys and humans was started by Dr. Blombery. After his return to Australia in December 1979, it was continued by Mrs. Gordon and Mr. Sherman. Over 50% of MHPG is converted to VMA, and about half of urinary VMA is derived from MHPG, indicating that urinary MHPG is not a reliable index of brain norepinephrine metabolism (Drs. Blombery, Kopin, Ms. Gordon, Drs. Ebert and Markey). The kinetics of the distribution and metabolism of MHPG reflected by plasma levels is in progress. These studies will include examination of the norepinephrine metabolites, DHPG, MHPG, and VMA, in plasma and alterations with postural change, stress, neuropsychiatric disorder and drugs.

The effects of phenylethanolamine-N-methyltransferase (PNMT) inhibitor in monkeys and man are being examined. After infusion of deuterated methionine, norepinephrine (NE) and epinephrine (E) are O-methylated to normetanephrine (NM) and metanephrine (M). Conversion of NE to EPI is assessed by examining incorporation of deuterium labelled methyl group from methionine into urinary M. The effect of the PNMT inhibitor can be measured by the decrease in the amounts of N-methylated deuterium in the metanephrine. Mr. Oliver has developed a GLC-MS method for the assay of these compounds. By monitoring ions formed from either end of the NM and M molecules the relative rates of O and N methylation and the amount and duration of the PNMT inhibition can be determined. This work is in progress.

Dr. Oddershede has joined the Unit since Dr. Blombery's departure. He is applying the laboratory procedures for norepinephrine and dopamine metabolites to the study of circadian rhythms of catecholamine metabolites in human urine and will apply these procedures to studies of stress, activity and drug administration in normal subjects or in patients with neuropsychiatric disorders.

Annual Report of the Laboratory of Developmental Psychology

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Marian Radke-Yarrow, Ph.D., Chief

Summary

The work of the Laboratory of Developmental Psychology is devoted to the study of child development and behavior, the primary interest being in affective and social behavioral properties of children. This focus provides coherence to a program of research that includes diverse problem areas: studies of normal and psychopathological social and emotional development, the effects of rearing conditions on behavioral development, and the interface of biomedical and behavioral processes in child development. Because assessment of social and affective dimensions of behavior and environment poses very stubborn scientific problems, methodological research continues to be an important emphasis in the program. The choice of research problems is guided by their closeness to clinical and environmental issues affecting children. It is assumed that behavioral studies can gain much in basic knowledge and potential applicability by close interaction of laboratory, with clinical and field, research. Most of the research of the Laboratory makes use of multimethods and involves collaborative investigation among the staff in the Laboratory, with other Laboratories in NIMH and NIH, and with other institutions such as hospitals and schools.

Developmental Studies of Rearing

In most psychological theories of human development, the family environment in which the child is reared is considered a major contributor to the child's immediate and long term functioning. The conversion of this environment to scientifically identifiable, measureable, and manipulable variables is essential if knowledge is to progress with regard to the contribution of experiential factors to outcomes in children's problems, mental illnesses, and well-being. Investigation has been seriously hampered by the lack of good techniques of measurement.

Substantial gains have been made in the Laboratory over the past ten years in developing methods for investigating child rearing. These are methods involving laboratory and naturalistic techniques (Drs. Yarrow and Waxler) which expand the range and depth of what is measurable in parental rearing and parent-child interactions. The methods make it possible to investigate problems that have not been immaculately studied by verbal report approaches or by usual experimental analogue paradigms of rearing. In the new methods, data are obtained by training parents to make systematic observations and to record (dictate) their observations close in time to the occurrence of intrafamilial behavioral events. Parents are supervised as data collectors. This data resource is combined with laboratory procedures which consist of repeated laboratory observational sessions with parent and

child. The laboratory conditions simulate a home environment which permits and requires the carrying out of childrearing functions and daily routines. In the sessions, there are standard, but natural, events that involve the parent in physical care of the child, stimulation, techniques for control and discipline, and affective interaction. The laboratory conditions also impose on the parent the kinds of usual demands that compete with giving full attention to the child. This research paradigm has applicability to the study of many questions of rearing influences and to the assessment of social and emotional behavior of children. With these methods it becomes possible to observe immediate and more remote causative environmental factors that contribute to pathological and to healthy affective and social development. A series of studies using this general strategy is now underway. The studies focus on affective dimensions of rearing, affective communication between parent and child, and parental control techniques.

In one study, by Dr. Leon Kuczynski, "reasoning" and "power assertion" as methods of controlling children's behavior are investigated. A longstanding assumption is that reasoning (the provision of explanations for commands and prohibitions) promotes long term changes in child behavior and facilitates the development of self-control, while "power assertion" (unexplained commands, punishments, rewards) promotes short term changes and hinders development of self-control. This view of discipline may be inadequate since recent findings suggest that the effects of reasoning depend on the nature of the explanation given. Data are being collected, on children of various ages, on the kinds of reasoning mothers use, the circumstances for which parents primarily rely on use of force in controlling behavior and those in which they use reasoning, and how discipline, observed over time, relates to the acquisition of specific aspects of self control. Only a longitudinal analysis of discipline will permit researchers to go beyond the study of immediate effects of specific control techniques to a consideration of long term influences and additive effects of discipline.

In a completed study of the control techniques used by mothers of 1-1/2 and 2-1/2 year old children, Drs. Waxler and Yarrow found that techniques that are effective in influencing children's later behavior are a combination of a clear cognitive message (explanation or demonstration) regarding the consequences for the other person of the child's own behavior, and a communication of intense feeling and commitment on the part of the parent. Neither nonaffective explanations nor power-assertive commands, alone, were effective.

Dr. Mark Cummings extended analyses of these data to an examination of an affective aspect of the rearing environment, namely, affection and anger expressed between parents in the presence of the child. He found that, in the second year of life, children are clearly detecting and discriminating emotional exchanges between parents, and that they often react directly toward the parents during incidents of anger and affection, by expressing jealousy, imitation, attempts to punish or comfort one or both parents. Repeated exposure to interparent anger increased the likelihood of negative emotional behaviors in the two year olds.

Also in the child rearing series and using the research strategy described, mothers who are selected on the basis of diagnosed psychological disorders and normal mothers are studied with their young children (Drs. Michael Chapman, Carolyn Waxler, and Marian Yarrow). In this research we are attempting to identify how parental pathologies are manifested in the rearing role, the nature of affective communication patterns between parent and child, and the children's emotional learning and development.

One project in the Laboratory goes outside the family rearing environment to examine the effects of a disruptive life experience that is imposed on large numbers of children in middle childhood. Dr. Editha Nottelmann is beginning a study of children in transition from elementary school to middle school and to junior high school. Many developmental and social problems of children appear at this time. The intersection of the demands of new environments with the developmental demands and issues of late childhood and adolescence is investigated. In this study the effects of the environmental discontinuities (in adult supervision, peer structure, etc.) on children's interpersonal relations and self concept are of special interest.

Developmental Studies of Prosocial and Antisocial Behavior

Research continues on the development of empathy and prosocial behavior in children. Empathy, the ability vicariously to experience another person's feelings, in earlier investigations in the Laboratory, has been documented as present in the second year of life and possibly earlier. The relation between empathy and developmental changes in cognitive abilities is the subject of a study by Drs. Waxler, Chapman, and Yarrow. Thirty children beginning at 12 months of age are tested periodically until 24 months to detect the child's ability to distinguish self from other person, and to understand properties of the physical world (object permanence test). In the same period, empathic emotional expression and prosocial behavior are being assessed. It is hypothesized that certain parallel developments in empathy and cognitive development take place in this period, however, the kind of interdependence of early cognitive and affective behavior is not known. In another study, these investigators are examining the interrelationships among cognitive, affective, and behavioral attributes in children from 4 to 11 years. Substantial response sampling is used with laboratory and naturalistic data. Findings show that performance on standard tests of perspective taking ability is not predictive of children's demonstrated inferential abilities in social interactions or of their prosocial behavior.

The issue of individual consistency and continuity in affective response tendencies is addressed in a longitudinal study (Drs. Waxler and Yarrow). Children observed in the second and third years of life were observed again in the seventh year. Individual patterns of response in the nature of emotional arousal and in the mode of reacting to emotional distress were apparent in the early assessments. These individual patterns show considerable stability into the seventh year. The assessments at follow-up were made independent of baseline knowledge, and were based on experimental and laboratory data.

Until this past year, the developmental work has been exclusively on prosocial behaviors of children. This year Dr. Mark Cummings has begun research with young children on the etiology of problem aggression. This work follows a strategy similar to that used in studying the development of prosocial behavior. Many data points over time will be obtained. The interrelations of developing aggression and developing prosocial responses will be considered.

Studies of Psychopathology

This domain of studies has been part of the Laboratory program for the past four years. Research has been directed mainly to the development of descriptive instruments for the assessment of children's behavioral competencies and disorders. This work has been under the direction of Dr. Thomas M. Achenbach. The Behavior Problems assessment instruments have been tested in a wide variety of outpatient and residential settings, with various clinical populations. Versions of the instruments have been developed for use by diverse informants, including parents, teachers, clinicians, trained observers, and children themselves. Data have been obtained on relations among ratings by the various informants. Data have also been obtained on changes in behavioral disorders and competencies following treatment. An interview survey of 1400 randomly selected parents of normal children was conducted to gather normative data and to permit assessment of differences in child behavior related to clinical status, age, sex, race, and socioeconomic status.

This research has been extended into a new phase; namely, the application of these instruments and findings to the interface between clinical and epidemiological questions. This is intended to provide a more comprehensive picture of the distribution and course of children's behavior disorders. For the past year, Dr. Achenbach has been on a work assignment at the University of Vermont to carry out the beginning phases of this work. The Vermont area has been chosen as a site because it offers children's mental health services that are centralized in a few public agencies. These individual mental health centers will use the NIMH instruments as part of their routine clinical assessment procedures, thus obtaining uniform data on all referred city and rural children. With a centralized computer facility to which the centers are linked, the centers will enter the raw data via their own terminals and immediately receive scored behavior profiles for clinical use with their clients and for research, evaluation, and survey purposes. The goal is a centralized case register system that can provide a complete regional clinical-epidemiological picture. It is anticipated that this research facility can serve ideally for child mental health assessment, information, and research. Dr. Jerome Blue, who has recently joined the Laboratory, is working with Dr. Achenbach in this program of research.

Laboratory research in the area of psychopathology has extended into another area, as indicated earlier. It is concerned with the functioning of severely affectively disturbed persons in the role of parent and with the consequences for children. Adults with affective illness who are on

pharmacological treatment are primary rearing agents, often despite serious behavioral deficits and disorders. The research is focused on the ways in which parents' symptomatology affect the care of the child. Rearing variables (parents' sensitivity-insensitivity to the child's needs, their methods of teaching and control, and their moods and emotions), are studied in relation to the child's affective and social characteristics. Drs. Waxler and Yarrow, in collaboration with members of the Biological Psychiatry Branch and the Clinical Psychobiology Branch, are studying a small group of depressed parents and their children. The families are studied in the laboratory and in the home, from the child's first birthday into the third year of life. Families have been observed and assessed at close intervals over this period. Although this research is still in the process of data collection, aberrant rearing functioning is clearly evident and there are extremes of ignoring, of love-withdrawal, and of over-protectiveness, as well as abuse and insensitivities to the child's signals and needs. The stressed children respond in divergent ways, but with individual predictability. There are social and affective changes in the children over the 18 months that they have been observed. This work is now being extended to become part of the series of investigations of affective aspects of normal and psychopathological rearing described earlier.

In two further projects, unfavorable rearing conditions are being investigated. Dr. Velma LaPoint is completing a study of the experiences of children whose mothers' are incarcerated. The rearing environments and the behavioral correlates in these children are compared with those of children from identical neighborhoods whose mothers have been involved in similar offenses but have been placed on probation. Related to this project, a conference of researchers and practitioners was conducted by Dr. LaPoint in which the problems of parents and children in relation to criminal justice were the subject of discussions.

In the second project, physically abused children are being studied. Here the conditions precipitating abuse and the consequences in the abused children will be investigated. This research has just been initiated by Drs. Penelope Trickett and Elizabeth Susman.

Biomedical-Behavioral Studies

Over the past four years, the work of a number of investigators in the Laboratory has been devoted to research on the interaction of biomedical problems and behavioral development. Biomedical conditions selected for study have been those in which developmental deficits are a high probability, or in which there is chronic and extreme psychological stress: long term nutritional deficiencies in young children, childhood cancer, premature births, and juvenile diabetes.

Chronic moderate malnutrition which affects vast numbers of children has received relatively little research attention, and specifically unexplored are its effects on the affective and social dimensions of children's behavior. However, information from animal research, clinical observation, and findings on attentional and cognitive deficits suggest functional disorders such as affective disturbance, problems in impulse control, and impaired social

functioning. These leads were investigated in two parallel studies of children, carried out by Drs. David Barrett and Marian R. Yarrow, focusing on the relation between nutritional deficits at different periods of early life and social-emotional functioning at school age. One is a study of urban children in the U.S.; the other, of rural children in Guatemala. Assessment of undernutrition and health history was accomplished through a battery of measures including anthropometry, nutrition and health history interviews, and blood analyses. Psychological and behavioral measurements include tests of sensorimotor and cognitive performance, and three standard half-day sessions in which the child's social interaction with peers and adults are measured. The sessions (experimentally planned, yet experienced as naturally occurring events) were designed to test a range of social interaction patterns, affective responses, and problem-solving behavior.

The results, to date, indicate that, relative to better nourished children from the same locales, children who have suffered nutritional deficiencies in early childhood begin school with a range of social and emotional, as well as sensorimotor and cognitive, deficiencies. They are less interactive with the environment, have less control of impulses, are less socially responsive, and show some affective withdrawal. These effects may well mediate the cognitive consequences of childhood malnutrition. Deleterious effects on sensorimotor processes, particularly coordination, are also indicated. Individual differences in socioeconomic variables, including housing, parent occupation, and mother's education, were not related to either the nutrition-health measures or to the behavioral outcome variables. Analyses of the paired studies will be continuing through the coming year.

A recent project, also in nutrition-behavior interaction, is part of a study initiated in NIH-NIAMDD, a developmental evaluation of children who as infants received Neo-Mull-Soy formula as their primary source of nourishment. A number of children on this diet were found to exhibit physical and behavioral abnormalities, a "failure to thrive" type syndrome. Analysis of the formula revealed that it was deficient in chloride and that many of the infants fed this formula were suffering from metabolic alkalosis and chloride and potassium deficiencies. Changes to a properly balanced formula improved their condition. There is some question, however, whether or not any permanent damage was sustained. These infants are being studied for a five-year period to investigate the possibility of long-term effects from this dietary deficiency. The project will involve extensive investigation by an interdisciplinary team. Dr. Howard Moss of the Laboratory of Developmental Psychology is responsible for the behavioral evaluation, which includes measurement of intellectual development, temperament, and interactions of mother and child.

In an earlier study carried out by Dr. Sarah Friedman, preterm infants, a group at risk for intellectual and behavioral deficits, were assessed on auditory, visual, and tactile responsiveness and on temperament. Drs. Waxler and Friedman have extended this research to include an investigation of the bilirubin levels in these preterm infants. Bilirubin levels in preterm infants are often elevated. It is hypothesized that a significant portion

of the diverse, adverse sequelae of preterm births is related to the neurotoxin bilirubin which damages regions of the brain. The purpose of this study is to determine if the amount of impairment in motor and sensory performance in preterms and if the altered affect expression evidenced in the infant cries are associated with concentrations of bilirubin in the blood. This research is in progress.

The preterm and full term infant cries have been further studied as stimuli to caregivers, to determine whether and how differences in cry characteristics influence reactions to the infant. The claim has been made that the premature cry is aversive and as such may elicit less good caretaking. The findings in this study do not fully support this position. Cries of preterm and full term infants can be discriminated by mothers, and, in general, the preterm cry is responded to more negatively. However, these cries, perceived as extreme distress, also elicit empathic responses from some adults, and, hence, may be assumed to be the signals for more attentive caregiving. The "aversive" premature cry cannot, therefore, be interpreted as having a single, detrimental effect on caregivers.

Again going back about four years, Drs. Elizabeth Susman and Howard Moss have been involved in collaborative studies with investigators in the National Cancer Institute. One of the studies concerned with central nervous system treatment (cranial irradiation and intrathecal chemotherapy) effects on the cognitive and perceptual functioning of children with leukemia found deficits in a patient sample compared with sibling control groups. This work is now being replicated in a prospective study. Children in remission from leukemia are evaluated on the same battery of psychological tests as in the first study. They are tested just prior to prophylactic CNS treatment and are reevaluated one year later to determine if learning disabilities or diminished intellectual functioning have resulted. The healthy siblings of the leukemics from the first study are used as a control group.

The purpose of another project by Dr. Elizabeth Susman was to investigate processes whereby child and adolescent patients and their families cope psychologically with the life threatening illness of childhood cancer. The patients were participants in an NCI study designed to test the utility of a protected environment (Laminar Air Flow room) for reducing infections while receiving experimental, intensive chemotherapy. Data collection methods included observational, interview, and rating procedures. Changes in behavior while receiving chemotherapy do not always follow changes in physiological status, as measured by white blood cell counts. The high variability among patients in social behavioral changes was related to aspects of caregiver and family interactions with the patient. While children's age is a good predictor of cognitive understanding of their disease, mothers' emotional reaction to the disease is a predictor of their emotional reaction to the disease.

The study of juvenile diabetes is appropriate as a model for understanding the interactions of biomedical and psychosocial factors. Two studies are continuing in this area. The main focus of one study by Dr. Moss is on

identifying stabilized behavioral patterns in response to the disease, and ascertaining factors which have led to the kinds of response patterns adopted. Particular attention is given to whether a child develops a passive-helpless orientation or attempts active-mastery. The children studied are between 11 and 18 years of age who have had juvenile onset diabetes for three years or longer. A matched control group of healthy children is also included. Interviews, standardized tests, and school and medical records are used.

The second project, by Dr. Beatrix Hamburg, involves comparisons of (1) metabolically labile, insulin-dependent adolescent diabetics, (2) non-labile diabetics, and (3) non-diabetic, non-obese individuals. For many years diabetes was viewed as a single hormone deficiency syndrome in which the disturbances of glucose metabolism could be reversed by exogenous insulin administration. Recent advances have established the multifactorial control of glucose regulation. The most prominent of the regulators that are known to increase blood sugar are also significantly responsive to stress. These include: growth hormone, glucagon, catecholamines, and cortisol. Recent studies of neuropeptides have shown that somatostatin can be a powerful inhibitor of both growth hormone and glucagon. The role of other neuropeptides in glucose metabolism is less clear. It has also been shown that physiologic increments of glucagon, epinephrine, and cortisol have synergistic actions. This work suggests that stress hyperglycemia cannot be ascribed to a single factor but is a consequence of the interactions of several regulators. It is hypothesized that there are significant individual differences in diabetic patients' patterns of biomedical response to stress which systematically relate to whether the patients are labile or non-labile. Some clinical approaches to the treatment of labile diabetic patients involve the assumption that the lability is due to interpersonal stress reactions stemming from family functioning. This model is examined in the present study by assessing biomedical and behavioral responses to interpersonal stress during an experimental procedure involving the family, to emotional but noninterpersonal stress, and to the stress of exercise. It is hypothesized that labile diabetics have aberrant biomedical patterns of response to stress but that these patterns of response may be triggered by emotional noninterpersonal stresses as well as by those of an interpersonal nature. The pattern of biomedical response to exercise stress is examined for evidence of aberrant metabolic response as a possible characteristic of labile patients.

In connection with the research on juvenile diabetes, Dr. Hamburg played a major role in planning and reporting a national conference on Behavioral and Psychosocial Issues in Diabetes, sponsored by four NIH Institutes, NIMH, and the National Diabetes Advisory Board.

In the past year, the professional staff of the Laboratory has undergone considerable change. Five Staff Fellows joined the Laboratory and all have quickly become involved both in the ongoing research programs and in initiating new studies. The Fellows are Drs. Blue, Cummings, Kuczynski, Nottelmann, and Trickett. Also, Dr. Elizabeth Susman who had been a Fellow in 1976-77 has returned to the Laboratory.

Annual Report of the Laboratory of Neuropsychology

National Institute of Mental Health

H. Enger Rosvold, Ph.D., Chief

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The resignation of Dr. Patricia Goldman, Chief of the Section on Developmental Neurobiology, is a real loss to the NIMH research effort. Her research program was not only unique, unparalleled in scope and intensity, but also provided a mould for many investigators in the field. At this time there will be no attempt to continue the Developmental research as a program; instead, developmental questions will be asked of other research. However, the Nursery will be maintained at a functional level, thus providing a shortened lead-time for other programs of the Laboratory. It is expected that one of these will deal with learning and memory as a major area of inquiry, and resources of the Developmental Neurobiology Section will be used to mount a strong program in this area.

The scope of the research has been broadened by developing collaborative efforts with other laboratories not only at NIH but also at several other centers of neuropsychological research. The following list reflects the variety of these efforts and as well how advantage is taken of the unique characteristics of these laboratories and their senior investigators. (1) Dr. David Klein (NICHD) Role of suprachiasmatic nucleus in pineal-melatonin rhythms in monkeys. (2) Dr. Mark Perlow (NIMH) Effect of pinealectomy on melatonin levels in monkey. (3) Drs. Louis Sokoloff and Christian Gillin (NIMH) 2-DG during sleep in monkeys. (4) Dr. Richard Wyatt (NIMH) Fetal brain tissue transplants in monkeys. (5) Dr. Robert Wurtz (NEI) Role of superior colliculus in eye movement in monkeys. (6) Dr. Monte Buchsbaum (NIMH) VER's to sine wave gratings in monkeys. (7) Dr. William Overman (U. of North Carolina) Cortico limbic mechanisms in memory for all four of these projects. (8) Dr. Lawrence Squire (UC San Diego). (9) Drs. Lawrence Weiskrantz and David Gaffan (Oxford). (10) Dr. Eiichi Iwai (Tokyo Metropolitan Institute of Neurosciences).

Cerebral Mechanisms in Object Perception and Memory

The cortical visual pathway

In a series of collaborative studies with Gross at Princeton, we delineated the functional pathway through which inferior temporal (IT) neurons receive their visual information. The method entailed measuring the defects in the visual receptive fields of single IT neurons after selective cerebral lesions or commissural transections. As we had predicted from our earlier

ablation studies, the subcortical pathway from superior colliculus through pulvinar to IT cortex turned out to be unimportant for the receptive field properties of IT neurons. By contrast, the cortical pathway from striate through prestriate to IT cortex proved to be essential, since interruption of this pathway by a unilateral striate removal or by section of the forebrain commissures eliminated the corresponding visual input to IT cells

We have now demonstrated this functional pathway autoradiographically using the [^{14}C]2-deoxyglucose method developed at NIMH by Sokoloff and his group. In collaboration with Kennedy in Sokoloff's laboratory we have found by comparing a blinded and a seeing hemisphere in the same monkey that the visual system can be clearly traced from the striate through the prestriate to the inferior temporal cortex on the basis of differential hemispheric uptake of the 2-deoxyglucose during visual stimulation. The studies were carried out in awake monkeys presented with visual patterns either in a rotating drum or in a discrimination apparatus. In the latter case, the animal performed the discrimination for water reward using the hand opposite the blind hemisphere. In both situations reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate projection but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. (Side-to-side differences were also seen subcortically in the temporal lobe as well as in collicular, pulvinar and parietal regions; these additional findings are relevant to later sections of this review.)

The prestriate relay

The results strongly support the view that the visual discrimination functions of IT cortex depend on the information it receives from the striate cortex via a relay in the prestriate. Yet one source of data has repeatedly raised serious problems for this conception of a corticocortical pathway, namely, the results of prestriate ablation studies. In a striking demonstration of this problem, two monkeys with massive prestriate removals who were impaired in the retention of a difficult visual pattern discrimination were gradually retrained by a method of approximation and were then given bilateral inferior temporal ablations in a second-stage operation. The supposition was that if the initial prestriate removal had produced a disconnection of inferior temporal from striate cortex, then the slow pattern discrimination relearning should have been achieved without the participation of inferior temporal cortex, and, consequently, removal of this cortex should not disrupt the relearned habit. As it turned out, however, the inferior temporal ablation produced a complete reinstatement of the deficit in both monkeys. The solution to the puzzle posed by this and other similar studies in the literature emerged from the following series of experiments.

To test directly the possibility of preserved neural transmission despite massive prestriate removals, an electrophysiological study of inferior temporal neurons was undertaken in animals prepared with such removals limited to one hemisphere. If the prestriate removals had produced a striate-temporal disconnection, inferior temporal neurons in the intact hemisphere should have had strictly contralateral visual fields, while inferior temporal neurons in the hemisphere with the prestriate ablation should have had strictly ipsilateral fields; that is, the results should have duplicated those obtained previously after unilateral striate removals. In fact, however, the receptive fields of approximately 25% of the neurons sampled did not fit this prediction.

In the normal animal, stimulation in a given hemifield will activate about 80% of inferior temporal neurons. By contrast, in the animals with left prestriate removals, stimulation in the right hemifield activated about 25% of inferior temporal neurons. Thus, on the one hand, the massive prestriate removals did greatly reduce the visual input to inferior temporal neurons - in fact, by more than two-thirds (80% to 25%) - thereby accounting for the severe deficit that such lesions produced in pattern discrimination relearning; on the other hand, the removals did not completely eliminate striate input to inferior temporal neurons (25% as compared with 0% after striate removals), thereby accounting for the ultimately successful retraining by approximation that was achieved in the animals with such lesion.

Even with this electrophysiological evidence, however, the conclusion that behavioral sparing resulted from sparing of remnants of the prestriate relay was only inferential. To demonstrate the preserved pathway directly, a neuroanatomical experiment was undertaken in two additional monkeys with massive prestriate removals who had also been successfully retrained by approximation on the pattern discrimination. More than a year after the prestriate removals, one of these animals was given a second-stage operation in which the entire occipital lobe posterior to the initial lesion was removed from one hemisphere, and the brain was examined for anterograde degeneration by the Fink-Heimer technique. The critical question was whether or not degeneration would appear in the inferior temporal cortex ipsilateral to the occipital lobectomy. Since striate cortex does not project directly to the inferior temporal area, the presence of degeneration here would indicate that the lobectomy had destroyed prestriate-relay tissue spared by the initial lesion, a finding that would account for the functional sparing that had now been demonstrated both behaviorally and electrophysiologically. In fact, terminal degeneration did result in the ipsilateral inferior temporal cortex - specifically, in the ventral part of area TE0. In addition, degenerating material was found in an unexpected area, the floor of the caudal portion of the superior temporal sulcus, which turned out to be a second spared route through which visual input could reach inferior temporal cortex. This was demonstrated in the other animal of the anatomical experiment, who was given a second-stage lesion in just this portion of the superior temporal sulcus of one hemisphere, again more than a year after the

initial prestriate removal. Terminal degeneration was found in the ipsilateral inferior temporal cortex of this case also - specifically, in the dorsal part of area TEO.

The foregoing series of experiments thus made clear that while the massive prestriate removals had severely disrupted the prestriate relay, they had not totally abolished it. Spared prestriate remnants that continued to serve as effective functional relays were directly demonstrated anatomically. To determine the source of these prestriate remnants, we turned to a study of the cortical efferents from all parts of striate cortex.

Striate projection system

One series of monkeys was prepared with unilateral lesions of lateral, posterior, or medial striate cortex, areas representing central, peripheral, and far peripheral vision, respectively. Collectively the lesions included all of area 17 with little or no invasion of area 18; their brains were processed for terminal degeneration by the Fink-Heimer procedure. In a second series, selected striate sites were injected with tritiated amino acids, and the brains processed for autoradiography; representations of the injection sites included the center of gaze and eccentricities ranging from 4 to 25° in either the upper or lower visual field. The results indicate that striate cortex projects to at least three separate and topographically organized visual areas within prestriate cortex.

The largest projection field is a circumstriate cortical belt which corresponds remarkably close to area OB of von Bonin and Bailey. It completely surrounds area 17 along with the 17-18 border except at the representation of fixation. Within this visual area, the representations of the upper and lower visual fields are entirely separate. Progression from central to far peripheral vision is represented: a) in the lower field, by a progression into the posterior bank and depth of the lunate sulcus, medially along the surface of the buried annectent gyrus into the parieto-occipital sulcus; and then rostrally along the upper lip of the calcarine fissure; b) in the upper field, by a progression into the inferior occipital sulcus, ventromedially into the occipitotemporal and collateral sulci, and then rostrally along the lower lip of the calcarine fissure. A second, smaller projection field is located in area OA along the caudal portion of the superior temporal sulcus; here, progression from central to far peripheral vision is represented by a progression down the posterior bank of the sulcus and continuing along its floor. Finally, a third, even smaller projection field, also located in area OA, begins in the anterolateral part of the annectent gyrus and extends forward to occupy the depth of the lateral bank of the intraparietal sulcus. Despite its small size, evidence of a complicated topographic organization within this field raises the possibility that it may contain more than one visuotopic map.

These three projection areas thus constitute all the possible prestriate routes through which striate output might reach inferior temporal

cortex. Undoubtedly, second and third-stage prestriate relays also participate in the striate-temporal pathway, although the exact location and topographic arrangement of these further relays remain to be delineated. But even from the evidence pertaining to the first stage alone, we can understand why attempts to produce striate-temporal disconnection by prestriate removal have repeatedly failed. All prestriate lesions to date have consistently spared varying extents of tissue that receive direct projections from posterior and medial striate cortex, the parts representing peripheral vision. These prestriate areas are the medial portion of area OB, the floor of the caudal portion of the superior temporal sulcus, and the depth of the most caudal portion of the intraparietal sulcus. Clearly, it was the sparing of this tissue that accounted for the continued, though reduced, transmission of visual information to inferior temporal cortex that had been demonstrated not only behaviorally but also electrophysiologically. Thus, the present anatomical findings provide a coherent explanation for a large body of seemingly paradoxical results regarding the effects of prestriate lesions. That is, just as "peripheral" striate cortex can mediate pattern discrimination in the absence of "central" striate cortex, so can "peripheral" prestriate cortex substitute for "central" prestriate cortex in this function.

Now that the total system of striate efferents has been delineated, the effects of completely disconnecting temporal from striate cortex can finally be investigated. Such disconnection should be achievable by removal of just the three striate projection fields that have been described, without inclusion of any other prestriate tissue. In addition, we are trying to determine whether even small prestriate lesions might disconnect IT cortex from corresponding parts of the visual field by testing visual discriminations confined to those fields in monkeys that have been trained to maintain fixation. In view of the visuotopic organization of the striate projection zones in both area OB and the caudal part of the superior temporal sulcus, even limited lesions within these prestriate zones should yield severe visual deficits provided the animals are forced to use the part of the visual field corresponding to the area damaged. To monitor fixation accurately, we have completed the design and construction of an infrared, corneal-reflection device with the aid of the Resources Services Branch.

Bilateral visual integration and stimulus equivalence

According to the unit recording studies referred to at the outset, nearly two-thirds of IT neurons receive information from both hemispheres (i.e., from both the left and right visual half-fields). We have therefore proposed that IT cortex plays an important role in stimulus equivalence across the visual fields. This is the phenomenon in which a stimulus is recognized as the same regardless of its retinal position, even if the different retinal loci project to different hemispheres. To test our hypothesis, we prepared monkeys with bilateral IT ablations combined with section of the optic chiasm, and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control

monkeys (with either IT lesions alone or chiasm section alone), who can perform immediately with the second eye any discrimination that they have learned with the first, the experimental monkey had to learn each discrimination anew with the second eye. These results provide strong evidence that stimulus equivalence across the visual half-fields depends on the convergence of their projections onto single IT neurons. We are planning to test a further prediction from this hypothesis, namely, that IT lesions will prevent transfer of a visual pattern discrimination from one part of a visual half-field to another. The method involves training monkeys whose maintained fixation is being monitored as described earlier to discriminate a pair of patterns in one quadrant of a visual half field and then test them for transfer to the other quadrant. Transfer should occur, as before, only if IT neurons are present to mediate the spatial convergence of visual information. In a related electrophysiological experiment we will test whether single neurons are indeed activated by their preferred stimuli irrespective of stimulus position in the visual field.

Not only TE but all visual areas within the cortical visual pathway are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO as well as TE receive their contralateral input via the splenium and anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we have attempted to localize and to quantify the contribution to vision made by the commissural systems. In a collaborative experiment with members of the Laboratory of Cerebral Metabolism, we measured rates of local cerebral glucose utilization (LCGU) throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract section alone. The 2-deoxyglucose method was applied one month postoperatively in awake rhesus monkeys actively viewing visual patterns. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemi- spheres of the two groups.

From the autoradiographs of each brain, representative sections were chosen at 1 mm intervals throughout the extent of the cortical visual pathway. Weighted averages of LCGU for the entire extent of each visual area were then calculated from these sections by means of a computerized image-processing system. In the intact hemisphere there was a progressive decline in LCGU along the cortical visual pathway from a high of 66 in area OC to a low of 47 $\mu\text{moles}/100\text{g}/\text{min}$ in anterior TE. This sequential decline in the intact hemisphere was the same for both the animals with tract section plus commissurotomy and those with tract section only. There were also no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere (ranging from 40% in OC to 60% in TEO). A difference attributable to visual input via the intact commissures was found in TE, how-

ever, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas in animals with tract section only LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. These results indicate that commissural inputs contribute more to the visual functions of area TE than to those of any other visual area and that commissural inputs alone may be insufficient to support visual function in areas TEO, OA, and the OC-OB border.

Object recognition

The spatial convergence upon single cells in area TE provides a mechanism that is essential for discrimination learning under normal viewing conditions. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite the fluctuations in fixation, distance, direction and angle of regard that lead to stimulation of different populations of striate and prestriate cells on different presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the effects of experience or training. We now have evidence that the IT cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Trained monkeys that are shown an object once will demonstrate that they recognize it as familiar several minutes later by avoiding it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus leaves a trace against which a subsequent stimulus presentation can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity, and the behavioral consequence described above ensues. The area in which the neural trace is first established turns out to be area TE, since removals here but not elsewhere abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, the TE cortex contains the traces laid down by previous viewing and these serve as central representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay, be renewed, or even refined, and new traces are added to the store. An experiment is now being designed to study these newly localized visual traces at the single-unit level. The question to be addressed is whether or not a neuron that at first responds equally to two similar stimuli can be induced to respond differently to them by training the animal to attend to their physical differences.

Object-reward association

The evidence that has been cited favors a sequential activation model for object vision. Information that reaches the striate cortex is trans-

mitted for further processing to the prestriate cortex, and from there to the IT area. Can this model be extended beyond vision per se to encompass stimulus-reward association learning? Translated into functional anatomical terms the question is whether visual objects might gain motivational and emotional significance through an interaction between the IT cortex and the limbic system. Some preliminary findings supported this possibility. Earlier we had shown that equally severe deficits in object discrimination reversal learning are produced by bilateral removal of IT cortex and by bilateral removal of the temporal pole and amygdala (TPA), a limbic region to which the IT cortex projects. To test whether the similarity in outcome reflects a truly functional relation between the two areas, we prepared monkeys with an IT removal in one hemisphere, a TPA removal in the other, and a transection of the anterior commissure. The effect on object reversal learning was the same as that of the bilaterally symmetrical lesions suggesting that section of the anterior commissure had disconnected the intact TPA region on one side from the intact IT cortex on the other. Section of the corpus callosum instead of the anterior commissure was ineffective. The results thus supported the proposal that the process of associating visual stimuli with rewards depends in part on a visual-limbic pathway with a link in the IT cortex.

We are now extending those studies to exploit this new evidence of a visual-limbic interaction. First, we undertook a neuroanatomical investigation using the Fink-Heimer technique to define exactly the amygdaloid projections not only from visual cortex, but from all the telencephalic sensory systems. The results support the following conclusions: 1. All sensory systems contain areas that project to the amygdaloid complex (the somatosensory system, tentatively so), but not to more central limbic structures in the basal forebrain and hypothalamus. Consequently, whatever influence the sensory systems have on emotional and motivational processes mediated by these more central limbic structures is likely to depend largely on relays through the amygdala. 2. Except for the olfactory system, the amygdalopetal projections arise only from the later stages of cortical processing within each sensory system, i.e., from the modality-specific association areas one or more steps removed from the primary sensory areas. Thus, the modality-specific cortical sources of the amygdalopetal projections, like their amygdaloid targets, are important links in the sensory-limbic pathways. These sources are: for vision, areas TE and ventral TG; for audition, anterior TA and dorsal TG; for taste, area IA; and for somesthesia, possibly areas IA or IB. The amygdalopetal sources thus occupy a limited territory that begins dorsally in the anterior insula and extends ventrally across the anterior temporal neocortex as far as the rhinal fissure. 3. Within the visual system, progressively heavier and more widespread efferents arise from successively later stages of the amygdalopetal sources. The posterior half of TE sends a moderate projection to the dorsal part of the lateral nucleus, the anterior half of TE sends a heavy projection to the dorsal parts of both the lateral and basal nuclei, and the ventral part of TG sends a heavy projection to the dorsal and medial parts of the lateral and basal nuclei and to the dorsal part of the basal acces-

sory nucleus. This pattern of progressive intensification and spread of the amygdalopetal projections applies also to the auditory system and probably to the other cortical sensory systems as well. The pattern suggests that a progressively greater influence on amygdaloid activity is exerted by successively more highly processed sensory information. 4. The efferents to the amygdaloid complex from the different sensory systems terminate in a dove-tailed pattern. The major amygdaloid targets are: for vision, the antero-dorsal parts of the lateral, basal, and basal accessory nuclei; for audition, the ventrocaudal parts of the lateral and basal accessory nuclei; for taste, the medial parts of the lateral and basal nuclei; and for olfaction, the cortical and medial nuclei. This pattern implies that each part of the amygdala is under the dominant influence of a particular sensory system.

The locus within the amygdala for the projection from the visual system was confirmed in the 2-deoxyglucose experiment described earlier. It would be of interest at some point to determine whether damage to just this part of the amygdala would produce the effects on visually mediated learning that have now been found after total amygdalectomy. This relates to the second way in which the visual-limbic interaction studies have been extended. Specifically, we have refined our behavioral assessment by replacing the discrimination reversal problem with a one-trial associative memory test analogous to the one-trial visual memory test described earlier. Whereas only IT lesions had impaired performance on the visual memory test, results with the associative memory test have indicated equivalent impairments after both IT lesions and lesions limited to the amygdala. Presumably, the impairment of the animals with IT lesions on the associative task was due to their basic recognition disorder found previously. The impairment in the animals with amygdala lesions, however, not being attributable to a recognition disorder, appears to reflect instead a disorder in object-reward association. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of IT cortex and the amygdala. An attempt will next be made to disconnect the amygdala from its IT input and then test the effects of this disconnection on associative memory.

Concurrently we will be testing directly whether the amygdala is involved in the establishment of stimulus-reward associations. The study involves measuring activity of single units in the amygdala while an animal performs a visual discrimination reversal. Positive evidence for our hypothesis would be provided by a finding that the discharge frequency of a given unit depends on the reward value of the stimulus (A+ vs A-) and not on its sensory quality (A vs B). The converse results, i.e., sensitivity to the sensory and not to the affective quality of the stimulus, would be expected of units in the IT cortex, for according to our model this tissue is an intrinsic part of the visual system and concerned with purely visual processing. Preliminary results have been gathered thus far on units in the IT cortex, and, as predicted, many of the neurons here discharge selectively to visual patterns - one cell preferring one subset of patterns, another

cell preferring another subset, and so on - whereas few if any of these neurons appear to code the reward value of the patterns. Whether such a code is carried by a higher proportion of neurons in the amygdala still remains to be determined.

Thus far, the cortical visual pathway has been discussed as though it transmits information only in one direction, from striate cortex forward to the limbic system. New evidence, however, suggests that the transmission may be reciprocal, such that the limbic system may modulate the activity of the visual system. This evidence was provided by a study that was carried out in collaboration with members of the section on Biochemistry and Pharmacology, BPB. Since some regions of cerebral cortex are known to contain a high concentration of opiate receptors, we undertook a detailed study of the distribution of these receptors across the entire cerebral cortex of the monkey. To accomplish this, rhesus monkeys were anesthetized with sodium pentothal before the brains were removed and dissected over ice into 50 areas which could be ordered into several functional hierarchies. Type I and Type II opiate receptors were then labelled under appropriate assay conditions with [^3H]naloxone and [^3H]D-ALA²,D-Leu⁵-enkephalin, respectively. The density of the stereospecific Type I opiate receptors increased in a gradient along the cortical visual pathway from primary sensory cortex (OC) to the ventral temporal pole (TG) which relays visual information to the deep amygdaloid nuclei. The relative opiate receptor density observed was 1 (OC), 1.3 (OB), 1.7 (OA/TEO), 2.5 (TE), and 4.2 (ventral TG). A similar progression was apparent in the auditory cortical system, with a 3-fold increase in Type I opiate receptor density in the lateral temporal pole (TG) over that in the supratemporal plane (TC/TB). Evidence for receptor gradients was also obtained in the somatosensory and motor systems and in the prefrontal cortex. This pattern of distribution was specific to the Type I receptor, the Type II receptor being relatively evenly distributed throughout the cerebral cortex, showing less than a 2-fold variation among the 50 regions. The gradients of receptor density that were observed for Type I opiate receptors could reflect a gradient of amygdalofugal projections back to cortex. This reciprocated connection thus offers a possible pathway through which affective states and emotional arousal could act as a gate for processing some aspects of sensory information in preference to others, in short, an emotion-induced selective attention.

Limbic memory mechanisms

In the process of investigating the role of various temporal-lobe structures in the visual memory of the monkey we have chanced upon a result that is particularly exciting because it may help to solve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed.

What we have found in our studies, both for visual recognition memory and for object-reward association memory, is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss ensues, a loss that is even greater than that produced by lesions of IT cortex. The discovery not only opens up a new possibility for resolving the discrepancy between clinical and animal findings, but also provides a new insight into the neural mechanisms of memory. Pursuit of this line of study is now a major part of our program. For example, two new experiments have shown that the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems. In both experiments, rhesus monkeys were trained preoperatively on a one-trial visual recognition task requiring memory of single objects for 10 seconds each, retrained on this task postoperatively, and then given a performance test in which their one-trial recognition ability was taxed with longer delays (up to 2 minutes) and longer list lengths (up to 10 objects). Their average scores on this performance test were compared with those of previously studied groups that had been given either separate amygdaloid and hippocampal removals (average score, 90%) or combined removals (average score, 60%).

In the first experiment, bilateral amygdalectomy was combined with unilateral hippocampectomy, and vice versa. Both groups obtained average scores near 75%, midway between those of the comparison groups. The results support the view that the amygdala and hippocampus are equally important for recognition memory and that there is a quantitative relationship between the amount of damage to the two structures and impairment of this function. The same conclusion could apply to man.

In the second experiment, bilateral amygdalectomy was combined with bilateral transection of the fornix, a major hippocampal-diencephalic pathway; and bilateral hippocampectomy was combined with bilateral transection of the stria terminalis, a major amygdaloid-diencephalic pathway. Again both groups obtained average scores near 75%. These results point not only to joint participation by the amygdala and hippocampus in short-term memory, but to a wider, limbic-diencephalic circuit underlying this process.

Coincidentally, it was found in the cortico-limbic anatomical study described above that the same cortical areas that give rise to separate sensory channels to the amygdala send efferents that converge upon the perirhinal and prorrhinal cortices, areas known to be a major source of input to the hippocampus. Consequently, both the amygdala and hippocampus can be activated by the same highly processed sensory information, a conclusion that may help to account for our finding that these two structures can substitute for each other in a mechanism for recognition memory.

It is important to point out that the memory failure that has been described does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with

the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though this list was presented only once every 24 hours. Thus, although animals with combined limbic lesions have an extremely short memory span on some tests, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. This paradoxical success implies the existence of an important memory mechanism outside the limbic structures of the temporal lobe. Further characterization of this non-limbic memory mechanism and localization of its neural substrate have become important goals of our future research.

A cortico-limbic pathway in somesthesia

The work described above elucidating a visual-limbic pathway has led to a search for analogous systems in other modalities. So far, a functional cortical pathway linking the somatosensory cortex with the limbic system has tentatively been established. Anatomical and electrophysiological evidence indicates that both area 5 of the posterior parietal cortex and the second somatosensory area (SII) are possible relays in a processing pathway connecting the primary somatosensory area (SI) with the limbic system. To evaluate these possibilities behaviorally, rhesus monkeys with bilateral lesions of either area 5 or SII were compared with normal controls on a battery of six somatosensory discriminations which required them to differentiate objects on the basis of texture, shape or size. Whereas animals with removals of area 5 showed no impairment relative to the controls in learning any of the tactual discriminations, monkeys with lesions of SII were markedly impaired relative to both of the other groups in learning all tasks except a gross size discrimination. Furthermore, despite learning the size discrimination normally, the SII group was impaired relative to the other groups in subsequent testing of size discrimination thresholds. Although tactual deficits following lesions of area 5 have been reported previously, deficits after such damage were found in the present study only when area 5 lesions were extended slightly rostrally, in a third operated group, to include the dorsomedial part of the hand representation of area 2. The combined results indicate that SII, and not area 5, is a critical station in a somatosensory processing pathway, and suggest that cortical areas to which SII projects may be further links in the pathway; this possibility was examined next.

Anatomical investigations have indicated a projection from SII to part of the insular cortex, and unit recording data show that neurons in the insular and retroinsular regions are activated by somatosensory stimuli. Therefore, a fourth operated group was prepared with bilateral lesions of the insular and retroinsular cortex, made accessible by removal of the

supratemporal plane. These animals were impaired in learning four of the tasks, including a roughness discrimination, but were unimpaired in size and roughness discrimination thresholds. Comparison of this profile of deficits with that following SII lesions suggests that the insular/retroinsular cortex is a higher order somatosensory processing area than SII.

The known projection of parts of the insular cortex to the amygdala suggests the existence of a sensory-limbic pathway for touch analogous to the one in vision. Just as visual sensory information is relayed to the limbic system through successive links of striate, prestriate, and inferior temporal cortex, a sensory-limbic pathway for touch may proceed from SI to the medial temporal lobe through serial links in SII and the insular/retroinsular cortex. To test this notion further, we are initiating metabolic, anatomical, physiological, and behavioral studies in somesthesia analogous to those described above for vision.

Cerebral Mechanisms in Spatial Perception and Memory

A second cortical visual pathway

The visual pathway described in the previous section is important for object vision, that is, for the analysis and coding of the physical dimensions of visual stimuli and for their identification through associations with other events, particularly motivational and emotional ones. There is a second major visual pathway out of the striate and prestriate cortex, however, and this one appears to play an important part in spatial vision, that is, in the analysis of the spatial location of objects and in the use of this information for the guidance of movement. The first pathway is directed ventrally to the temporal and frontal limbic systems. By contrast, the second pathway is directed dorsally and appears to provide access to the motor system. The evidence in support of this dichotomy of cortical visual pathways and the reasons for categorizing the second one as primarily spatial rather than purely visual in function come from recent experiments in our laboratory on the parietal and frontal lobes.

We had found earlier that bilateral posterior parietal lesions produced severe impairments on a spatial test in which the animal was required to choose a response location on the basis of its proximity to a visual "landmark". The effective lesions were large, invading not only inferior parietal but also dorsolateral and medial prestriate tissue. To test whether the responsible damage might have been to the prestriate rather than to the parietal cortex, new experiments were performed with more selective lesions. The results failed to reveal any localization within the larger region, and indicated instead that each part contributes to the function equally. The findings thus give added support to the view that the inferior parietal lobule, rather than being a modality-specific tactual association area as was once thought, is probably a multimodal area with a significant part played by vision. To test for the dependence of this area on visual input, a crossed-lesion study was undertaken. A posterior parietal lesion

in one hemisphere was combined serially with a total striate removal in the other, and these were followed by callosal transection. Animals trained on the landmark test were severely impaired by the crossed lesions alone (performed in either order), and once recovered they were only mildly re-impaired by the callosal lesion. The results suggest that the inferior parietal cortex, like the inferior temporal, depends heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive a heavy ipsilateral visual input by way of the corpus callosum. Thus, each posterior parietal area may be organized largely as a substrate for contralateral spatial function, and this could account in part for the symptom of contralateral spatial neglect that has so often been reported after unilateral parietal injury.

In another experiment, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal functions, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for the parietal just as for the temporal area, the striate inputs to these two regions appear to be organized differently: relative to inferior temporal cortex, posterior parietal cortex receives a greater contribution from inputs representing both the contralateral and the peripheral visual fields. These differences, which are seen also in the receptive field properties of posterior parietal vs. inferior temporal neurons, presumably reflect differences in the cortical processing required for spatial vs. object vision. Incidentally, a direct demonstration that the posterior parietal cortex is visually related was provided by the 2-deoxyglucose experiment described in the previous section.

The spatial functions of the parietal area have been categorized on the basis of our studies as "allocentric", where spatial location is defined in terms of referents external to the animal. Another form of spatial perception is "autocentric", where location is defined with reference to the animal's own body. Evidence is accumulating that this form of spatial orientation is dependent on dorsal prefrontal cortex, and that impairment in autocentric localization after dorsal prefrontal damage is the basis for the frontal animal's impairment on spatial delayed response tasks. (The dissociation between an allocentric and an autocentric spatial mechanism derives in part from our finding that parietal and prefrontal lesions each impair one mechanism but not the other.)

Crossed-lesion experiments like those described above have shown that the dorsal prefrontal cortex also depends on striate input for its visual

spatial function. Evidence that the function is selectively spatial comes from a recent experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests. These included delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the cortex in the principal sulcus quickly reattained criterion performance, in sharp contrast to their notoriously poor performance on spatial visual memory tests. In another experiment we obtained evidence that autocentric spatial localization is probably not a purely kinesthetic function. On a test that required precise judgments of the extent of head or hand movements, prefrontal lesions alone produced little or no impairments. Since it is thus possible to spare kinesthetic discrimination ability while at the same time severely impair spatial perception, it is clear that the two abilities must be at least partly independent. At the same time, other evidence was obtained that with sufficiently large frontal lesions, which included both dorsal prefrontal and dorsal premotor cortex, a severe kinesthetic impairment could be produced. The findings thus point to a substrate for kinesthesia that is close to, yet separate from, the substrate for spatial perception. Indeed, it is reasonable to suppose that the system for autocentric localization depends on input not only from vision, but from kinesthesia and many other modalities, and, further, that the dorsal prefrontal area, like the posterior parietal, is a center for convergence of multimodal information organized in terms of some spatial coordinate system. The hypothesis suggests a new direction for electrophysiological studies of these dorsal cortical association areas.

Activation function of the spatial-processing system

It was noted above that the outputs of these dorsal association areas may be directed toward the cortical motor system and thus permit spatial guidance of movements. Disconnection experiments involving the spatial and motor systems, like those described earlier involving the visual and limbic systems, would provide a direct test of this proposal. Indirect support for this notion came from an unexpected source. In the previous section the evidence was reviewed showing that visual discrimination habits depend on both the cortical visual system and ventral limbic structures. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex, including that in the dorsal "spatial" system, also play a significant role? To examine this question we restricted vision to a single hemisphere (by visually deafferenting and disconnecting the other hemisphere), and then removed all the cortex from the seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies (performed in collaboration with members of the Section on Clinical Psychophysiology, BPB) and from the subsequent histological examination of the brains.

To determine whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other, animals were prepared as before but with the forebrain commissures left intact. Yet these animals too were blind, indicating that the blindness is not a disconnection effect and suggesting further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. The analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Brodmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferior parietal, insula and superior temporal areas, did produce blindness in three monkeys for about a month. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of the neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing neglect.

Monkeys prepared with the standard lesion were behaviorally blind for periods ranging from 25 to more than 400 days. The extreme variability in the duration of the blindness has not only been puzzling but has impeded further analysis of the phenomenon. New studies indicate that this variability can be eliminated and a predictably long-term effect produced in two different preparations.

First the original findings suggested a species difference that has now been confirmed with additional animals given the standard lesion. Thus, whereas a total of four rhesus monkeys (Macaca mulatta) have recovered from blindness after a median of only 28 days, none of four cynomolgus monkeys (Macaca fascicularis) has yet shown recovery despite a median postoperative survival period of more than a year.

Second, the rhesus monkey also will show prolonged blindness if the standard lesion is expanded to include most of the limbic cortex. Three animals prepared in this way have thus far remained blind for a median period of one year with no signs of recovery. The limbic lesion alone will not cause this effect as evidenced by two monkeys with such a limbic ablation that demonstrated accurate visual guidance immediately after surgery.

We had proposed on the basis of the previous evidence that the blindness results from a failure of visual processing within the intact

visual system due to the loss of an essential activation normally provided, directly or indirectly, by the territory included in the ablation. The newest findings strengthen this hypothesis by rendering highly unlikely the alternative possibility that the blindness reflects some transient state such as surgical shock or diaschisis.

To investigate the basis of the blindness we have begun single-unit recordings in the blind animals. Striate cortex was studied in five monkeys prepared as above to produce chronic blindness, except that the optic tract was left intact in two of the animals to provide control hemispheres. The recordings were made while the monkeys were anesthetized with nitrous oxide and immobilized with pancuronium bromide. Implanted wells and head holders were used to minimize possible sources of pressure or pain.

We isolated 244 units from the hemispheres made 'blind' by the ablation, and 83 units from the 'seeing' intact hemispheres. Of the units from the blind hemispheres, 53% were moderately or highly responsive visually as contrasted with 95% of those from the seeing hemispheres. The loss of visual responsiveness was related to a consistent histological finding of inadvertent damage to optic radiations representing primarily the lower visual field, in that there was a strong positive correlation between a cell's visual responsiveness and the vertical position of its receptive field. For example, at more than 50° below the horizontal meridian, only 12% of the units were moderately or highly responsive, whereas at more than 50° above the horizontal meridian, 71% of the units were moderately or highly responsive.

There was no difference between the blind and seeing hemispheres either in the proportion of responsive cells with oriented receptive fields (69% and 68%, respectively) or in the proportion of oriented cells with complex-type receptive fields (71% and 63%, respectively).

We concluded that: 1) although many units respond poorly or not at all in the striate cortex of the blind hemispheres, there appear to be enough moderately or highly responsive units to support vision, particularly in the upper fields; 2) moreover, visual information is not only reaching striate cortex but is being processed there as indicated by the normal proportions of cells with oriented and with complex-type receptive fields; 3) the loss of visual processing or disconnection of the efferent visual pathway responsible for the blindness must be occurring at some point beyond striate cortex.

It appears from these results that the dorsal "spatial" system normally provides an activating input that is necessary for the sensory systems to function properly, and that this is in addition to its contribution to spatial mechanisms. For the animals that eventually do recover their use of vision, they show permanent impairment in the ability to guide their limbs to visual targets; that is, they then show the expected spatial-motor disconnection. Further studies dealing with both the spatial and the activating

functions of the posterior parietal and dorsal prefrontal areas are now underway. In addition, we are currently pursuing the anatomical circuitry underlying the proposed activation. Our initial results suggest that the pulvinar nucleus in the thalamus may play an important role.

Role of the pulvinar

We had previously found that striate projections to the prestriate cortical area OB are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to area OB. In subsequent studies, we found that there is also a precise visuotopic organization of striate projections to the pulvinar, indicating the existence of two sources of striate input to area OB that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via the pulvinar. This parallel system of inputs to area OB thus provides a possible mechanism by which activating signals (e.g., from dorsal cortical areas to the pulvinar via midbrain structures) acquire visual field specification, that is, a mechanism which tells the organism where in the field to attend. Although this hypothetical circuit may apply for all sensory systems, for the visual modality our data suggest that only the inferior and lateral nuclei of the pulvinar are involved. This conclusion was inferred from the anatomical material from two series of rhesus monkeys in which striate projections to the pulvinar were mapped. Future anatomical studies will investigate the sources of midbrain input to PI and PL, as well as the organization of afferents to the pulvinar from other sensory modalities.

Subcortical Mechanisms Related to Frontal Lobe Functions in the Monkey

The Substantia Nigra and Prefrontal Cortical Interrelationships

Activity in this project was suspended for a time due principally to staff changes. However, the investigation is now well on its way again, and tooling up is almost completed. The summary from last year's project summary is repeated here to provide a sense of continuity in the program as a whole. The intimate anatomical and biochemical relationship between the caudate nucleus and the substantia nigra suggests that they may subsume similar cognitive functions. It has been established that lesions placed in the caudate nucleus of the rhesus monkey impair performance on delayed alternation performance. However, massive lesions placed in the substantia nigra of a pilot animal had little if any effect. It becomes important therefore to establish the generality of this negative finding, and to explore what significance for behavior there is in biochemical and anatomical similarities in these structures.

Annual Report of the
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Herbert Weingartner, Ph.D., Acting Chief

Research activities in the Laboratory of Psychology and Psychopathology have focused on psychological and psychobiological functioning in unimpaired individuals and the study of impaired individuals in the following areas:

1. Psychobiology of cognitive processes.
2. Individual differences and the study of heredity and environment in psychopathology particularly in schizophrenia.
3. Autonomic nervous system activity and attention.
4. Psychology and biology of depression.
5. Neuropsychology views of dementia.

Psychobiology of Cognition

Research projects have been designed to explore and define the psychobiology of cognitive processes in man. Studies have examined the psychobiological processes that define the processing, learning, and storage of information, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Some of the components of cognitive processes that have been examined include attentional determinants, aspects of short-term memory, the consolidation of information in long-term memory, the kinds of state and trait specific cognitive strategies used in the elaboration and transformation of information, organization in memory, "effortful" vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve experiences that they have stored in memory.

These research efforts have utilized two broadly conceptualized strategies for defining the psychobiology of cognitive processes. One is to contrast the effects of different treatment strategies on different components of cognition, such as on information storage, consolidation, encoding and retrieval. These studies include attempts to systematically alter cognition through: (1) pharmacological manipulations, such as with cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and (2) by behavioral manipulations that alter arousal/activation, stimulus attributes (altering encodability) and types of stimulus processing strategies subjects use to process information. These studies are carried out in the unimpaired subject, as well as in patient groups with different forms of psychopathology. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of enhanced or disrupted cognition?

The rationale for the second kind of strategy for researching the psychobiology of cognition is to compare different forms of cognitive failures seen in different psychiatric and neurological syndromes. Would the disrupted cognition seen in some psychopathological states be qualitatively and quantitatively different and related to specific changes in central nervous system activity? For example, how are the amnesic/cognitive impairments seen in Huntington's disease, Korsakoff syndrome, Alzheimer's disorder, different, and how might such differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, discriminating between different forms of cognitive impairments is necessary for both adequate diagnosis and effective treatment, such as the cognitive disruptions that are part of depression as opposed to that produced by a progressive dementia. Frequently, depression is associated with and a response to a progressive dementia, and the cognitive impairment that is evident is a joint product of both of these forms of disorder. Some studies that are part of this project have also investigate the therapeutic potential of various psychoactive drugs and behavioral treatments as these might attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity and learning disability syndromes in children, depression, mania and the schizophrenias. Each of these studies that make up this research project can be seen as clinically relevant, as well as pertinent for understanding unimpaired cognitive processes. Studies have been designed and completed in the following areas:

1. Behaviorally defined mechanisms that alter components of cognition

Characteristics of stimuli such as: (a) imagery, (b) emotional arousing attributes of stimuli, (c) information presentation rate, (d) mode of processing, (e) language vs. pattern information, and (f) types of learning, have been studied in relation to its effects on acquisition (learning), strength of learning, retention, and components of information retrieval. These studies have examined the structure of cognitive processes in normal controls, as well as in patient groups (depressed patients, hyperactive children, learning disabled, patients suffering from various forms of dementia).

2. Pharmacological alterations (enhancement and disruption) of cognitive processes

These studies have contrasted the cognitive enhancing effects of cholinergic agents such as arecoline, physostigmine, lecithin treatment with those of amphetamine and neuropeptides. These studies have been conducted in both normal subjects, as well as in patients (hyperactive children, Alzheimer's disorder depressed patients). Would different kinds of psychoactive drug treatments that produce qualitatively different central nervous system responses produce systematically different changes in cognitive processing? That is, would enhancements or disruptions of cognition occur through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernible?

In some instances, drugs that might disrupt aspects of the acquisition of information may enhance some other stage of cognitive processing (e.g., alcohol

administered prior to, or just after acquisition). These stage and process-specific effects of drugs that alter cognition can be used to better define a psychobiology of information processing in man. This research has also involved attempts to find clinically useful strategies for altering disrupted cognition in man.

3. State-dependent learning

This area of research provides a useful framework for exploring: (a) the qualitatively unique manner in which events are stored (encoded and retrieved from memory), (b) mood state specific strategies for processing experience and remembering past events in memory, (c) qualitative changes in cognition in response to psychoactive drugs, (d) contextual factors as determinants for defining the nature of trace events in memory, and (e) individual differences in susceptibility to state dependent or dissociative mood/drug effects.

4. Cognition and mood

Studies have included research of mood-related changes in: (1) the brain lateralization of cognitive functions, (2) arousal and activation and its role in information processing, and (3) the encoding and retrieval of events in normal mood and while depressed or manic. This research has examined how patients with disturbances in mood process information in a mood-state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive processes. Other research has explored the degree to which effortful processing of information is compromised in depression. The cognitive impairment in depression may be determined by psychobiological changes in motivational states.

5. Memory consolidation

This research has focused on the psychobiological events that follow the acquisition (storage) of information and well before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory, and the processes that might further sustain, enhance, or disrupt stored trace events that already are part of memory. The rate of decay of information and the susceptibility of information to interference may be important determinants in defining what is available and accessible in recall once information has been stored in memory. Disruptions in consolidation may contribute to the cognitive failures in the dementias.

Drugs that disrupt memory and learning may do so by altering biological operations that succeed acquisition or learning. Likewise, drugs e.g., neuropeptides, may enhance aspects of learning and memory by facilitating the consolidation of learned information.

6. Cognitive disruptions in the dementias

Studies have examined and contrasted the specific mechanisms and structure that defines cognitive failures in different neuropsychiatric

populations. Studies of components of cognition that are affected in Huntington's disease have been systematically explored and contrasted to cognitive processing in Korsakoff's psychosis, Alzheimer's disorder, and other neuropsychiatric disorders. These studies have attempted to discover whether much of the cognitive failures seen in these syndromes may be due to encoding (acquisition failures), unreliable access to information which is in memory, interference factors, and disruptions in consolidation. These studies have attempted to uncover the mechanisms or determinants of the cognitive failures in different forms of dementia. Understanding the structure of such impairments is considered essential in conceptualizing and evaluating therapeutic drug trials that might attenuate these cognitive impairments. These studies have lead to studies which have explored how various psychoactive drugs might prove useful in reversing aspects of the cognitive changes that define a particular dementia, while also providing information about the underlying psychological mechanisms which may be disrupted in each of these syndromes.

7. Mechanisms of cognitive impairments that determine forms of learning-disabilities

Studies have been designed which investigate: (a) forms and incidence of various kinds of learning disabilities in children, (b) the structure and characteristics of learning disabilities in these children, (c) cognitive process and determinants that produce impaired learning in these children, and (d) potential strategies for their remediation.

Major Findings: Studies of psychobiological determinants of cognition in unimpaired subjects

In a number of experiments we have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and its retrieval. Arecoline, physostigmine, and lecithin treatments produce enhancements in the storage and retrieval of information. Scopolamine treatment, which disrupts cholinergic activity, produces an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine-induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated patients with Huntington's disease and other forms of dementia.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in an undrugged state, and afterwards are treated with arecoline, there is a comparable facilitation in recall, and is like the facilitated learning and recall that occurs when subjects are pretreated with Arecoline prior to learning.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input processing under drug state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of

studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition in depressed patients, hyperactive children, normal children, and normal adults. Amphetamine also induces a change in state which serves as a state-specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent learning and state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine, and the cognitive disrupting effects produced by scopolamine vs. lithium has served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

Alcohol, when administered after the processing of information produces an enhancement in recall when later recall is tested in the unintoxicated state. This paradoxical cognitive facilitating effect of alcohol, administered during a consolidation phase of memory, highlights the differentiated mechanisms and components that make up information processing, memory, learning, and retrieval.

Some behavioral strategies have also been used to investigate the specificity of cognitive processes as these are involved either separately or together in the acquisition, elaboration and amplification, trace maintenance in memory, and retrieval. The emotional properties of stimuli seem to amplify or strengthen what is learned or stored in memory in a manner similar to that produced by drug treatments such as amphetamine. Reinforcement following information processing also acts to strengthen events already in memory storage. Other behavioral determinants, such as word imagery, presentation rate, familiarity, or stimulus structure appear to alter how much is learned, rather than the strength of learning, in a manner similar to the effects of increased cholinergic activity.

Recent findings from behavioral studies of cognitive processes in unimpaired individuals have differentiated two kinds of cognitive operations. One kind requires effortful processing and results in effective encoding of events and the establishment of viable memory traces. The other kind of process appears to be relatively "automatic" requiring little effort, and is not easily disrupted in man. The mechanisms of these processes appear to be different. Drugs that alter one kind of processing do not seem to alter the other kind of cognitive operation (e.g., alcohol impairs recall of items (effortful processing), but not their frequency of occurrence (automatic processing). This scheme for describing the structure of cognition (thinking, learning, remembering) in man will be investigated further.

Drug strategies for altering cognition in impaired patients

Systematic individual differences are apparent in the effects of cholinergic agents in altering cognition in man. For example, slow learners benefit more (learn more effectively) following administration of cholinergic drugs, while "good" learners are less affected by these drugs.

In therapeutic trials in patients with progressive idiopathic dementias (e.g., Alzheimer's disease), we have observed a small but reliable cognitive

facilitation. This has been evident following arecoline administration and in response to the administration of a combination of cholinergic substances. This work is being expanded and capitalizes on two other sets of findings: (a) one that defines the mechanisms of memory failure in senile dementia, and (b) the specific effects of neuropeptides on cognition.

Studies of state-dependent learning in children treated with amphetamine have demonstrated striking individual differences in dissociative effects. Fifteen children have been studied under four different experimental conditions including: (a) learning under drug condition (LD), recall under drug condition (RD), (b) or learning following placebo treatment (LP), recall following placebo treatment (RP), both of which are congruent learning recall conditions in contrast to (c) LD, RP, or (d) LP, RD, both of which are disparate learning recall conditions. Somewhat more than half of these children show clear dissociative effects, so that when learning occurs under one condition, e.g., following amphetamine treatment, but memory is tested in the undrugged state, then memory appears to "fail" particularly for poorly processed items, or those that are recalled without benefit of cues or prompts. These findings point to individual differences in the amphetamine response with respect to the form of the cognitive effects in hyperactive children. These dissociative effects also have clear and important implications for the classroom functioning of stimulant drug-treated hyperactive children.

Studies that define cognitive changes in clinical populations

HD patients fail to consistently remember information which is, nevertheless, in memory store. They also demonstrate retrieval failures which are due to inadequate encoding of information during acquisition. They fail to use slow presentation rate, semantic encoding, imagery, and other attributes of stimuli to more effectively and elaborately encode information. Some drug and behavioral treatments may prove helpful to normalize this encoding disruption.

Alzheimer's patients also fail to "appreciate," learn and, retrieve experiences. These patients have access to appropriate semantic structures in memory but fail to use these structures in organizing experiences so that they are memorable.

In contrast to HD patients, Alzheimer's patients appear to show a small but measurable cognitive response (improvement) following cholinergic and amphetamine treatment. Recall of organized information is most facilitated while rote-like learning seems unaltered. It is, as yet, uncertain whether the cholinergic response is qualitatively different from that seen following amphetamine treatment. These patients have also been treated chronically with a cholinergic drug and have appeared to show a temporary improvement in cognitive functions, but this enhancement is not a sustained drug response. Strategies for defining cognitive changes in Alzheimer's disorder and potential treatment strategies are relevant issues in the study of normal aging processes.

Recent studies testing the cognitive changes that follow treatment with vasopressin-like compounds (DDAVP) may provide us with some new strategies

in the treatment of dementias. We have shown that DDAVP produces a marked and sustained cognitive enhancement in depressed patients, as well as in normal controls. The form of that cognitive enhancement appears through an alteration of organizational mechanisms which may be particularly relevant for the kinds of disruptions in cognition seen in Alzheimer's patients. Studies of the structure of cognitive changes in response to neuropeptide treatment should prove helpful in defining biological mechanisms that are important for those cognitive processes involved in the organization and elaboration of information in memory.

Recent completed cognitive studies of depression have shown that these patients demonstrate learning memory failures when they are required to organize information, but show no such deficit when organization is provided and present. That is, when information is presented in an organized fashion, then depression-related cognitive deficits are not apparent. In some respects, these cognitive disruptions are similar to those seen in Alzheimer's disorder. These patients also demonstrate qualitative changes in how they encode events and the strategies by which they recollect previously learned experiences (state-dependent learning). Some of these effects may be related to alterations in lateralization of cognitive processes.

Studies of cognitive processes in children have demonstrated some of the specificity of cognitive disturbances that are apparent in hyperactive and learning-disabled children. Hyperactive children appear to learn (store) about the same amount of information as normal controls (under experimental conditions). However, unlike controls, retrieval of that information is highly dependent on the presence of strong environmental cues or prompts at the time remembering takes place. Both normal and hyperactive children respond with enhanced cognition following amphetamine treatment. However, those aspects of cognition that are enhanced in these different children are qualitatively different. The facilitation of cognitive functions in the hyperactive child is one which would not readily translate into more effective school performance, since amphetamine increases learning and recall of superficial characteristics of information processing. This is in contrast to an amphetamine-induced increase in learning and recall of meaningfully processed information in the normal child. It, therefore, appears useful to integrate behavioral retraining strategies along with stimulant treatment to effectively help the hyperactive child.

An integrative system for exploring the psychobiology of cognitive processes

Ongoing studies have provided findings which permit us to examine (a) how specific aspects of cognition are altered by drugs that affect activity in different parts of the central nervous system, and (b) different forms of neuropathology and psychopathology lead to different alterations in the cognitive processes that normally would lead to successful appreciation, acquisition, and retrieval of experience. The findings from the studies described here have suggested a pattern of relationships between various component cognitive processes. These patterns have emerged from pharmacological, behavioral, and clinical studies and findings that suggest that distinctive psychobiological mechanisms are involved in the organization, encoding, consistent retrieval,

and consolidation of information in memory. Different behavioral-neurochemical determinants may alter the strength of trace events in memory, their accessibility, and the amount of stored experience in memory.

Autonomic Nervous System Activity and Psychopathology

Research in this area has focused on autonomic nervous system activity (ANS), attention, and information processing in psychopathology and the study of biological and psychological correlates of these processes in humans. ANS activity is measured by simultaneous recording of several peripheral indices such as skin conductance (sometimes bilaterally), heart rate, and skin temperature under conditions of rest, presentation of simple stimuli (orienting response), and task performance which may vary in stressfulness. Attention and information processing are measured by various reaction time and perceptual speed techniques.

A study of a heterogeneous sample of schizophrenics has confirmed the findings of higher indices of resting ANS arousal and lower tonic and phasic ANS reactivity in these patients. Skin conductance level--a commonly used index of ANS arousal--was also not higher than normal in the patients (despite much higher levels on other arousal indicators). Effects of various pharmacological and nonpharmacological treatments on ANS are also being studied. The effects of renal dialysis on ANS activity in seven of these patients is being evaluated. Under active dialysis as compared to placebo the patients were more autonomically responsive to novel and/or aversive stimuli (e.g., 100 dB tones) suggesting that whatever substances the dialysis removed from the system, one or more have an inhibitory effect on autonomic "orienting" responses. Studies on affective disorder patients and on adolescent and adult obsessive patients, using methods similar to those used in some of the schizophrenic studies are being accomplished in order to investigate the diagnostic specificity of patterns of ANS activity. In addition, the effects of pharmacological therapy on these patients are being studied.

The effects of dextroamphetamine on ANS activity and attention have been studied in normal and hyperactive boys and in normal men given two dose levels. Marked attentional improvements and reductions in impulsive behavior on the drug were found in both groups of children. The ANS effects on both groups of boys were similar and suggested an increased specificity of ANS reactions to imperative or "demanding" stimuli and situations (more marked in the hyperactives), compatible with their improved attention. The data suggest that hyperactive children do not respond atypically to stimulant drugs. Male adults showed a decrease in the specificity of their ANS reactions to important stimuli when on the drug and also showed increased ANS "arousal" and slow habituation of the orienting response. These effects of amphetamine are similar to previous research findings in drug-free schizophrenics compared to normal subjects, suggesting possible ANS mediation of amphetamine psychosis and spontaneous psychotic episodes.

A similar study of the effects of acute doses of caffeine in children found that this drug improved reaction time, increased electrodermal indices of arousal and responsivity, but decreased tonic HR. Thus, this "stimulant"

drug which affects the benzodiazapine receptors has a different pattern of effects from amphetamine. A chronic study (2 weeks) of caffeine in children is in progress.

In two studies the effects of physical and psychological stress on plasma norepinephrine (NE) and ANS variables were tested. Patients with hypertension and orthostatic hypotension and normal controls were tested in one study, and normal men rated on "coronary prone" (Type A vs. Type B) behavior were tested in a second study. NE increased only during the physical stress, electrodermal measures mainly during the psychological stress, and cardiovascular variables responded to all stressors. Type B men, surprisingly, were higher on NE and heart rate at rest and gave larger reactions to the physical stress. Thus, there was a lack of association between several measures of ANS activity.

Genetic Studies, Individual Differences, and Psychopathology

The studies in this area have been directed at how hereditary and environmental determinants interact to influence the incidence and expression of psychopathology. A series of studies have shown that hereditary factors contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. The evidence thus far suggests that the mode of genetic transmission in schizophrenia is polygenic or a dominant single gene with polygenic modifiers.

Proposed research projects have attempted to: (1) try to discriminate gene carriers and nongene carriers with respect to personality and test variables; (2) compare the fate of gene carriers who are reared in the parental home and compared to those reared in adoptive homes; (3) compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; (4) find the incidence of schizophrenia spectrum disorder in a random sample of the population.

Studies of Depression

The premise of this project is that there are, in addition to the well-known mood changes, important alterations in perceptual and cognitive processes in major affective illness. Such symptomatology may have important implications for the pathophysiology of affective disease, and may provide significant contributions to delineating clinically and prognostically homogeneous subtypes of this condition. The present study is composed of nine investigations, looking at various aspects of the problem. They are aimed at documenting the nature and extent of cognitive and perceptual changes at the clinical level, and of investigating the degree and structure of such deficits in the laboratory. A summary of the current studies within this project follows.

Atypicality in Major Depressive Illness

The initiation of this project was based on observations of considerable heterogeneity of clinical presentation within the group of patients meeting

Research Diagnostic Criteria for primary, endogenous, and major depressive disorder. This clinical diversity may contribute significantly to the biological and prognostic heterogeneity within this group. To investigate the possibility of important subtypes, or of an important atypicality dimension within this group, rating scales were constructed to measure atypical depressive illness. The scale is designed to measure degree and type of divergence from a classical syndrome of discrete episodes of autonomous mood and vegetative symptoms.

Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive disorder. Ninety-one percent of these patients met criteria for endogenous depression as well. There were 21 men and 23 women; 28 patients were bipolar and 16 unipolar. In a preliminary analysis, global atypicality ratings on a zero to ten scale were derived by consensus of trained raters from the psychiatry, social work, and nursing services. Subsequently, the same group consensually rated patients on each of 24 items, to give a total scale atypicality score. Scale items assessed degree of autonomy of symptoms, vegetative signs, personality disorder, neurotic symptoms, thinking and perceptual disturbances, past course of illness, and premorbid adjustment.

Atypicality ratings derived by the two different methods were highly correlated ($r = .83$, $p < .0001$). Atypicality was characterized by relative lack of encapsulated episodes, disturbed interpersonal relationships, narcissistic entitlement, manipulativeness, and prominent anxiety and somatization. Atypicality scores were not related to severity of depression. Atypical patients were significantly younger than typical patients ($p < .002$). Bipolar I patients were significantly more typical than either bipolar II or unipolar patients ($p < .05$), however, the difference no longer reached significance when age was covaried out. Atypical patients were hospitalized significantly more often ($p < .02$) than typical patients, even though the typicals tended to be faster cyclers than the atypicals. Biologically, the typical group had significantly less variance in measures of MMPG metabolism as reflected in urinary MMPG ($p < .05$) and spinal fluid nor-epinephrine ($p < .03$). While the atypicals tended to have higher MMPG levels, they had lower platelet MAO ($p < .056$). Typicals were significantly more likely than atypicals to have an antidepressant response to sleep deprivation than atypicals ($p = .048$).

The preliminary data cited above suggest that atypicality may be an important factor within primary, endogenous depression. It is not yet clear whether atypicality is best conceptualized as a subgroup or as a dimension within depression. It may be that the atypical depressed patients are distinct from those patients that present with more typical depressive symptoms, and have a greater propensity for regressive episodes and hospitalization. It is notable that within our group, atypicality was represented by features of narcissistic character disorder and disturbed interpersonal relationships, rather than by schizoid trends or thinking disorders. There was an indication that the bipolar I group was highly typical, while the bipolar II and unipolar patients were equal in atypicality. Because age and polarity were confounded in our patient population, it was impossible to reliably test for an association between typicality and polarity.

Biologically, the trend toward higher MMPG and lower variance in norepinephrine metabolism can be placed in context of previous biological studies. Generally speaking, lower MMPG has been associated with more classical, and more severe forms of depression. While low platelet MAO has also been associated with more classical (bipolar) depression, it is also associated with a group of people within the general population who have high levels of personality disorder, particularly sensation seeking and sociopathy. Thus, within our group, atypical patients tended to have low MAO, while typical patients had either high or low MAO.

While the above data were gathered retrospectively by consensus, plans are now underway to rate subjects as they enter the hospital. Working with a fresh, common data base will allow higher reliability than retrospective consensus ratings. Information gathered in this way will enable us to examine correlates of varying types of atypicality, rather than just total scale scores. In addition, we are collecting data on course of illness and response to treatment after NIH hospitalization.

Psychomotor and psychosensory symptoms in affective illness. A wide range of behavioral and perceptual manifestations, many with localizing significance in the brain, have been described as concomitants of complex partial seizures. The literature and clinical observation suggest that there may be areas of overlap between these phenomena and symptoms of major affective illness. The purpose of the present study is to investigate the nature and degree of such overlap. At the present time, approximately 70 affective and epileptic patients have been studied. On the basis of preliminary data, it appears that the two groups are similar in incidence of psychosensory symptomatology; it forms a prominent part of the clinical picture in roughly 25% of both groups. Data will be analyzed to evaluate the relationship of symptoms to course of illness, drug response, and personality characteristics of the subjects.

Perception and recall of emotional and neutral stimuli in depression. It is a common clinical observation that affectively ill patients often seem highly insensitive to their own internal emotional state. It is also well known that depressed subjects perform more poorly than controls in a variety of memory tasks. The purpose of this study is to look systematically at how depressed subjects evaluate the emotional qualities of verbal material, how such evaluations change with changes in clinical status, and how they interact with the subjects ability to recall the material. Normal and depressed subjects rate words similarly as to high or low emotionality, although depressed subject's ratings of the high-emotion words tended to be lower, with greater variance than normals. Depressed subjects remember fewer words, both freely and on recognition, than normals. However, while in the normals there is no relation between emotionality ratings and memory performance, in the depressed group, the lower the ratings, the fewer words were recalled. Furthermore, the depressed group showed evidence of preferential storage and retrieval of high-emotion words. In the light of previous memory studies with normal and depressed subjects, this data suggests that the depressed group processes stimuli more shallowly than normals, and that sensitivity to emotional qualities of words is an indicator of ability to process deeply in the depressed, but not the normal group.

Hypothesis Testing in Depression. Preliminary results indicate that depressed subjects are unable to focus on a correct hypothesis in concept learning, as well as normals. That this is not due to inattention is suggested by the fact that they form as many coherent hypotheses as normals. Our current evidence also suggests that the deficit is not primarily one of memory on this task, since forcing subjects to adopt an appropriate strategy (without giving them any memory aids) significantly improves performance. Thus, depressed subjects appear deficient in ability to form useful problem-solving strategies. Such a deficit may relate to clinical phenomena such as depressed patients' overgeneralized thinking and feelings of being overwhelmed by problems.

Luria neuropsychological investigation in depressed, schizophrenic, and Alzheimer's patients. We are currently surveying depressed, schizophrenic, and Alzheimer's patients for deficits in higher cortical functioning, using the standardized Luria examination. Indications to date suggest left temporo-parietal dysfunction in the schizophrenic group, and global deficits, including visual perceptual dysfunction in the Alzheimer's. Data in depression is not yet sufficient to provide reliable indications. We are planning to compare Luria results with presence and type of brain abnormality as shown by C-T scan in all three groups. Intergroup comparisons will also examine possible distinct cognitive profiles in demented and psychiatrically impaired patient groups.

Cognitive and mood response to epinephrine in normal and depressed subjects This study has three major objectives. The first is to investigate the relationship between epinephrine-induced peripheral sympathetic arousal and performance on a verbal memory task in normal subjects. Effects on learning will be related both to pulse and blood pressure changes, and to subjective mood and activation status of the subjects, as induced by the drug. The second objective is to compare the effects of adrenalin on physiologic and psychologic factors in normal and depressed subjects. Although schizophrenic subjects have been shown to have blunted physiologic responses to epinephrine, the comparable study in major depression has not been done. It has also been shown that autonomic arousal and cognitive set combine to produce a subjective mood state in normal subjects. Since depressed populations are known to have altered cognitive processes, and appear clinically to show deficits in "emotional proprioception," mood response to adrenalin in this group is of theoretical interest. The third objective of the study is to investigate the ability of adrenalin, a peripherally acting drug, to induce state-dependent learning effects in normal and depressed populations. Drug induced state-dependent learning has been demonstrated for a variety of centrally acting drugs, but never with ones whose action is essentially peripheral. The study will also provide insight into the conditions necessary for the production of a dissociative state, in particular, the relationship between state-dependent phenomena, and physiologic, subjective, and behavioral changes induced in the subjects. All phases of the project involve intravenous infusions of the drug at a carefully controlled rate, with continuous cardiac and blood pressure monitoring. The initial phase of the study, involving normal volunteers only, has recently gotten under way. Early data suggest changes in learning and memory, level of activation, and anxiety level, but not other aspects of mood under the influence of the drug.

Lateralized hemispheric function in depression. There are presently two distinct parts to this study. The first involves an extensive review of the literature concerning hemispheric lateralization of mood-related behavior and emotional aspects of cognitive processing. Taken as a whole, the current literature suggests that while the right hemisphere appears predominant in the control of many aspects of emotional behavior and processing, there is also a division of function between the hemispheres, with the right hemisphere dominant for negative effects and the left dominant for positive effects. Evidence for these two types of lateralization comes from a wide variety of experimntal paradigms and subject populations, each providing a relatively small number of studies. It is clear from the literature that a great deal of confirmatory work must be done in normal, brain-lesioned, and affectively ill populations before the relationship of laterality to mood state can be understood.

The second part of this study involves lateralized tachistoscopic presentation of visual material to depressed and control subjects. The task involves the subject making "same" and "different" judgments on material that can be processed either linguistically (left hemisphere) or on the basis of form (right hemisphere). Differential performance of the two hemispheres will be compared in the two subject groups. This part of the study is currently in the final stages of technical adjustment, and data collection will be initiated soon.

Visual and olfactory perceptual changes in depression. This study was initiated in order to investigate whether cognitive impairment found in depression might in part be due to deficits at the level of sensory processing. Because of the intimate relationship of the olfactory system to limbic areas of the brain that are involved in regulation of emotions, the sense of smell was chosen for the initial phase of the investigation. Threshold detection level, and psychophysical judgments of intensity and pleasantness were obtained from three groups of subjects: depressed, hypomanic, and recovered patients, temporal lobe epileptics, and normal controls. Data on 27 patients, 15 epileptics, and 12 controls has been collected. There are no differences in detection levels of four odorants, two pleasant and two unpleasant, among the three groups. In their ratings of pleasantness and intensity, patients in all three states performed like normal controls. Epileptics, however, had supersensitivity to odor intensity and pleasantness in their ratings. This difference appears to occur at a later stage of evaluation rather than at the primary stage of olfactory sensitivity. To investigate the possibility of subtle changes in the depressed group that might have been missed, we are preparing a psychophysical scaling task for two olfactory and two visual stimuli. On the basis of this task, sensitivity and response biases of normal and depressed subjects can be carefully quantitated and compared.

Pharmacological Studies of Depression

This project covers three pharmacologic studies in depression using different classes of drugs, which interact with different neurotransmitter systems. Amphetamine, a drug with well-known activating and mood-altering properties, stimulates release of dopamine and norepinephrine. Clonidine

is an antihypertensive agent with central noradrenergic agonist actions. ACTH is a pituitary hormone with actions on both the adrenal cortex and receptors in the brain itself. Orranon 2766 is an analog of ACTH 4-9, a fragment of ACTH known to act on CNS receptors, but not on the adrenal. In the initial phase of the amphetamine study, 18 patients with unipolar and bipolar depression were given 20 mg intravenous amphetamine. The group response consisted generally of activation and mood elevation, although there was considerable heterogeneity in direction of mood change and degree of emotionality following drug administration. Bipolar depression was significantly related to mood elevation following drug. A report based on this phase of the investigation has been submitted for publication. At the present time we are analyzing blood samples drawn during the procedure to investigate correlations of biological response and mental status change. Changes in MMPG levels before and after amphetamine have been reported to correlate with both amphetamine and tricyclic antidepressant response; cortisol and growth hormone levels have also been reported to vary with certain aspects of amphetamine response. The present phase of the study will attempt to corroborate these findings, as well as to relate the reactive subtypes found in the clinical study to biological parameters.

Clonidine is a drug with known noradrenergic agonist actions. A significant portion of these actions is presynaptic. It has been demonstrated in animals, for example, that systemic clonidine infusions decrease firing of the locus coeruleus, a noradrenergic center in the brainstem. This center is involved in the mediation of activation and anxiety levels, and success in treating opiate withdrawal with clonidine is presumably due to the drug's rating action on its activity. Since there are indications of increased noradrenergic activity in mania and anxious depressions, we felt that the action of clonidine on these conditions would be of interest. The protocol involves intravenous administration of 2 micrograms/kg to depressed and manic patients. Mood and anxiety levels are monitored during the procedure; blood samples are drawn for measurement of noradrenergic metabolism as reflected in MMPG. In addition, levels of growth hormone, luteinizing hormone, and prolactin are being measured at intervals throughout the procedure. Initial data indicate a possible anti-manic effect, as well as changes in anxiety level in depressed subjects. Preliminary blood analysis suggests a possible blunting of growth hormone response to the drug, as compared to normal controls.

The studies of ACTH in depression is presently being completed. This research was designed to investigate the effects in depressed patients of ACTH, and an analog of the ACTH 4-9 fragment, a compound known to be active at sites in the brain (particularly hippocampus), but devoid of adrenocorticotrophic effects. Dependent measure included mood ratings, a verbal and visual learning battery, evoked potential studies, sleep studies, and blood assays of cortisol and norepinephrine. Data collection for the study has now been completed, and most of the results have been analyzed. Patients showed no drug effect on mood or memory performance. ACTH administration was found to disrupt REM sleep, while ACTH 4-9 disrupted slow-wave sleep. ACTH and ACTH 4-9 were found to have effects on the attentional response of subjects to lights and tones, as measured by average evoked response of the EEG. While ACTH enhances the attentional component of the AER compared to placebo, ACTH 4-9 diminishes it to the point of eliminating the usual attention-stimulus

intensity gradient. It is notable that the most prominent effects of these drugs appeared at the longest times after administration, raising the question for future studies of whether peak effects on mood and memory, if any, would appear in a later time frame than the one currently used.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1979 through September 30, 1980
Melvin L. Kohn, Ph.D., Chief

The focus of this Laboratory's research is studying the effects of the larger social environment upon individual psychological functioning and behavior. We have long since demonstrated systematic patterns of correlation between people's positions in the structure of the society and their self-conceptions, values, orientations, and intellectual functioning. Now we are engaged in longitudinal and cross-national comparative studies designed to elucidate the causal processes that produce these correlations. Three major efforts are underway: a long-term longitudinal study of job conditions and psychological functioning; cross-national studies in Poland and Japan designed to test the limits of generalizeability of our U.S.-based findings on the interrelationship of social stratification, occupational structure, and psychological functioning; and a longitudinal study of stress and coping. What we can say this year about all three efforts is tentative and incomplete, for we are in the middle of intensive data-analysis in them all. We can say with some assurance, however, that we are developing solid evidence about the precise processes by which the social environment affects important aspects of individual personality and behavior.

OCCUPATIONAL EXPERIENCE AND PSYCHOLOGICAL FUNCTIONING

The principal goal of this research is to assess the relationships between people's occupational conditions and their psychological functioning. The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a selected child.

One major purpose of the follow-up study was to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators

hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of wives was designed to ascertain whether occupational conditions affect men and women similarly. (On this, see the 1977-78 report.) These data should also enable the investigators to assess the effects of men's occupational experiences on their wives' psychological functioning and of women's occupational experiences on their husbands' psychological functioning, in each case taking account of the individual's own occupational experiences. The study of the children was designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, the major research efforts of the "occupations study" have been addressed to five objectives: (1) Using the longitudinal data for men, analyzing the reciprocal effects of job conditions and psychological functioning. (2) Using the data from both the men and their wives, analyzing the reciprocal effects of people's job conditions and the intellectuality of their leisure-time activities. (3) Again using the data from both the men and their wives, analyzing the relationships between housework and psychological functioning. (4) Beginning an assessment of the processes by which education affects children's psychological development. Finally, (5) beginning an assessment of the effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning.

1. Longitudinal analysis of the reciprocal effects of job conditions and psychological functioning. The intent here is a definitive assessment of whether, and if so the extent to which, job conditions affect men's psychological functioning and whether, and if so the extent to which, men's psychological functioning affects their job conditions. These analyses began (see the 1976-77 Annual Report) with an assessment of the reciprocal effects of one pivotal job condition--the substantive complexity of work--and one central facet of psychological functioning--intellectual flexibility. In that phase of the analysis, Melvin Kohn and Carmi Schooler demonstrated that the substantive complexity of work has a decided effect, essentially ongoing and continuous, on intellectual flexibility. Intellectual flexibility has a much more gradual, albeit in time even greater, effect on the substantive complexity of work.

The causal model was then expanded (see the 1977-78 Annual Report) to a more comprehensive assessment of job structure and intellectual flexibility. The substantive complexity of work is the single most important occupational determinant of intellectual

flexibility, but several other job conditions also affect and are affected by intellectual flexibility.

The next step in the analysis (see the 1978-79 Annual Report) was the application of the same causal model to each of a number of other facets of psychological functioning -- e.g., authoritarian conservatism, self-esteem, anxiety, and standards of morality. These analyses demonstrate the far-reaching effects of job conditions, not only on intellectual functioning, but also on self-conception and social orientation.

During this past year, Kohn and Schooler have attempted to develop a more general causal model of job conditions and personality, a model that simultaneously assesses several job conditions, intellectual flexibility, and several other facets of psychological functioning. This task has proved immensely difficult, primarily because some aspects of self-conception and social orientation are highly correlated with intellectual flexibility -- e.g., authoritarian conservatism is so highly correlated with intellectual flexibility that it is not possible with ordinary statistical procedures to estimate their effects on each other or their independent effects on other variables in the model. The investigators have pursued three distinct approaches to this problem, one unsuccessfully, another partially successfully, and the third at too early a stage of analysis to be certain, but with thus far encouraging results.

One approach, thus far unsuccessful, has been to develop new mathematical procedures for estimating structural-equation models involving linearly dependent variables. A second approach, which successfully deals with at least part of the problem, has been to develop a model of job structure, intellectual flexibility, and those aspects of self-conception and social orientation that are not so closely tied to intellectual flexibility as to pose mathematical problems in a causal analysis. In a sense, this is a model of job structure, intellectual functioning, and "non-intellective" facets of personality. This model validates what had previously been learned from the simpler models, confirming the reciprocal effects of job conditions and several facets of psychological functioning, thus demonstrating that the effects are not simply repeated illustrations of the same basic phenomenon. It also shows effects of the psychological variables on one another that are entirely plausible. There are two interrelated clusters of intra-psychic effects, one centering on trustfulness, the other on anxiety, with both affecting intellectual flexibility.

What makes this dual clustering especially intriguing is its implication that there may be two analytically distinct dimensions to personality (or, more precisely, to those aspects of orientation to

self and others measured in this study), which would be substantively interesting and potentially a way out of the linear-dependency problem. Kohn and Schooler hypothesized that one principal dimension of orientation is self-direction/conformity, a self-directed orientation centering around non-authoritarianism, holding personally responsible moral standards, and being trustful of others. They also hypothesized that a second principal dimension of orientation is psychic distress/well-being, psychic distress being measured principally by anxiety, self-deprecation, and lack of self-confidence. A confirmatory factor analysis, using the several facets of self-conception and social orientation as the indicators for two higher level factors, self-directedness and psychic distress, confirms these expectations.

The investigators are currently working on a causal model of job conditions, intellectual flexibility, self-directedness, and psychic distress. The results thus far are highly encouraging.

2. Job conditions and the use of leisure-time. The purpose of this analysis, being conducted by Karen Miller and Melvin Kohn, is to examine the relationship between people's job conditions and the intellectuality of their leisure-time activities. This past year Miller and Kohn expanded the measurement model of leisure-time intellectuality they had earlier developed (see the 1978-79 Annual Report), to include the intellectual level of the sports in which people actively engage -- this in addition to the number of hours spent watching television, frequency of attendance at plays, concerts, and museums, number of books read, time spent on hobbies, intellectual level of magazines read, and intellectual level of newspaper reading. Adding sports results in a better representation of intellectuality in the leisure-time activities of people at varying educational levels. The measurement models for men and women are very similar.

Causal analyses using the expanded measurement model of leisure-time intellectuality have thus far been done only for men. These analyses generally confirm the conclusions previously drawn about the effects of men's job conditions on their leisure-time intellectuality. In particular, the substantive complexity of work has by far the strongest effect of any of the occupational conditions: the more substantively complex is men's work, the more intellectually demanding is their use of leisure-time. The only real difference between the earlier findings and the present findings is that a wider range of job conditions is now shown to significantly affect leisure-time intellectuality: Routinization, time pressure, dirtiness of the job, risk of loss of job or business, and hours worked all have relatively small negative effects, while job income and heaviness of the work have relatively small positive effects on leisure-time

intellectuality. In the reverse causal direction, leisure-time intellectuality affects only the substantive complexity of men's jobs. Thus, men who engage in intellectually demanding leisure-time activities mold their jobs to be more substantively complex.

The investigators have not yet completed causal analysis of employed women's job conditions and leisure-time intellectuality. Preliminary multiple-regression analyses do indicate that there may be sex differences in the importance of particular job conditions for leisure-time activity. Position in the organizational structure -- i.e., ownership, hierarchical position, and bureaucratization -- appears to have considerably stronger effects on women's than on men's leisure-time intellectuality, while the substantive complexity of work appears to have stronger effects on men's than on women's leisure-time intellectuality. Causal analysis for women will show whether these sex differences hold under more stringent and precise statistical controls.

3. Housework and psychological functioning. On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to those of work conditions encountered in paid employment outside the home, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondent in taking care of the household. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household-work questions were asked not only of housewives, but also of working women and of men.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have developed measurement models of the basic conditions of work encountered in housework (see the 1978-79 Annual Report). Having created these indices, the investigators are now examining the relationships between housework and psychological functioning. The initial analyses have been limited to women. In these analyses, each facet of psychological functioning is treated as a dependent variable in a multiple-regression equation in which the independent variables are the "job conditions" of housework and relevant social background characteristics.

These analyses reveal striking relationships between the degree of self-direction exercised in housework -- in particular, its substantive complexity -- and several aspects of psychological functioning. There are also relationships between the pressures encountered in housework -- time pressure, heaviness, and dirtiness, for example -- and psychological functioning. In general, these relationships are similar to those previously found for paid employment. Just as with paid employment, women whose housework is

self-directed are more likely to be intellectually flexible, to have a positive view of themselves, to be open and flexible in orientation to others, and to value self-direction for their children. Women working under pressured conditions show signs of psychological distress such as anxiety, self-deprecation and ideational rigidity and are likely to value conformity rather than self-direction for their children. However, because of the strong possibility that housework may not only affect but may also be affected by psychological functioning, these multiple regression analyses provide only presumptive evidence that housework actually affects psychological functioning.

Causal models depicting the reciprocal effects of housework and psychological functioning are being estimated through the use of structural-equation modeling. These analyses -- not yet completed, but far enough along to permit cautious conclusions -- provide evidence that housework does affect psychological functioning and that the effects of housework on psychological functioning are generally greater than are those of psychological functioning on housework. These conclusions, though, are highly tentative, for much more analysis remains to be done.

4. Educational experience and children's psychological development. Karen Miller, Melvin Kohn, and Carmi Schooler's analysis examines the processes by which students' educational experiences, particularly the degree of self-direction they exercise in educational endeavors, affect their psychological functioning. The intent is to see whether the concepts and methods developed for the study of occupational experience can be applied with similar results to the study of educational experience. In effect, Kohn and Schooler have developed a theory of occupation as an educational experience. Now Miller, Kohn, and Schooler are attempting to apply the same concepts to schooling itself.

Relevant data were collected in the 1974 follow-up survey as part of the interview with children of the primary respondents. The interview schedule for these "children" -- now aged 13 to 25 -- contains a battery of questions about the current educational experiences of all the respondents still in school. The questions are designed to parallel those previously found to be potent for analyzing occupational experience, focusing on such dimensions of the educational experience as its substantive complexity, how closely it is supervised, time-pressure, and the like.

The project is in a preliminary stage of conceptualization, data-file creation, and exploratory analysis. Thus far, the investigators have developed measurement models of one basic concept, "closeness of supervision" by the teacher. The models prove valid for

students at all educational levels, from junior high school through college. Now in process is an attempt to create measurement models for the substantive complexity of schoolwork, models which may have to be tailored to particular educational levels. Still ahead is the major task of developing measurement models for other aspects of school experience and for psychological functioning. Only then can a systematic causal analysis of educational experience and psychological functioning be undertaken.

5. The effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning. Carmi Schooler, Melvin Kohn, and Ronald Schoenberg have barely begun this analysis. The intent is to determine the extent to which, and the processes by which, a married person's job experiences affect his spouse's values, self-conception, social orientation, and intellectual functioning, taking into account the spouse's own occupational experiences and social characteristics. There is some evidence (from Pearlín and Kohn's analyses of the Turin study some years ago) that a man's job experiences affect his wife's values, but the evidence is limited and based on rather primitive statistical methods. The present investigators intend a much more comprehensive analysis, not only of the effects of men's job conditions on their wives' values, but also of each spouse's job conditions on many facets of his partner's psychological functioning, using complex causal models. The inquiry has not yet gotten much beyond verifying the applicability of measurement models developed in other parts of the occupations study to the problem at hand.

CROSS-NATIONAL REPLICATIONS

The Polish replication. The main purpose of this inquiry is to examine the interrelationship of social stratification, job conditions and psychological functioning in a socialist society. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. The 1978-79 Annual Report reviewed their efforts to develop methods that assure cross-national comparability of indices. This year, the work has been principally addressed to one of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.?

In general, the investigators find that the relationships between social stratificational position and psychological functioning are

remarkably similar in Poland and the United States. For example, the correlations between social stratificational position and intellectual flexibility are 0.75 in Poland and 0.77 in the U.S., with education accounting for the lion's share of those correlations in both countries, but with occupational status and income also making independent contributions. Similarly, social stratificational position correlates 0.48 and 0.51 with parental valuation of self-direction in the two countries. Moreover, the relationships between social stratificational position and such aspects of social orientation as authoritarian conservatism, standards of morality, trustfulness and idea-conformity are also remarkably similar in the two countries. But, for reasons the investigators do not as yet fully understand, the relationships between social stratificational position and self-conception are somewhat different in the two countries. In particular, in the U.S., higher social position is associated with more self-confidence ($r = 0.22$), but in Poland the opposite is true (the corresponding correlation being -0.12 .)

Having established the similarity of the relationships between social stratificational position and intellectual flexibility, parental values, and social orientation for the two countries, the investigators are now pursuing the question, whether occupational conditions play as crucial a role in explaining the relationships in Poland as in the United States.

The Japanese replication. During the past year another major replication of the Kohn-Schooler survey was conducted, this one in Japan. The replication was carried out by Atsushi Naoi and Kenichi Tominaga of the Department of Sociology of Tokyo University, in collaboration with Carmi Schooler. The replication involved interviewing a probability sample of more than 800 employed Japanese men, using a questionnaire containing all the questions necessary for indexing the structural imperatives of the job and those aspects of psychological functioning measured in the American and Polish studies. This replication is of particular interest not only for the cross-cultural comparisons it will provide, but also because the structure of industry is rather different in Japan, with a much sharper cleavage between the primary and secondary labor markets than is true in the United States.

Data collection took place during the summer and fall of 1979 and the data were coded for statistical analysis in Japan during the 1979-1980 academic year. It is expected that data analysis will begin in October 1980, when Naoi is scheduled to come to the Laboratory as a Visiting Scientist.

SOCIAL-STRUCTURAL SOURCES OF STRESS

In the third major study, Leonard Pearlin has been examining the interrelationship of stress, coping, and psychological well-being. His research looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two times. In 1972 a sample of 2300 people, representative of the Chicago Urbanized Area were interviewed. These interviews sought information about three features of people's lives: the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; the coping strategies people employ in dealing with life-problems; and symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 people drawn from those originally interviewed. Many of the questions that had initially been asked about chronic role problems, coping, and psychological distress were repeated, thus providing longitudinal information about stability and change in these aspects of people's lives. Additional questions sought information about key life-events that occurred since 1972, both the many changes and role transitions that are normative aspects of the life-cycle and the more unexpected, non-normative disruptions of important roles and activities, such as divorce, serious illness, involuntary unemployment, and other life crises. It is the long-range goal of the research to reconstruct from this information those elements of social experience that importantly affect mental health and to identify those coping behaviors and social supports that serve as barriers to psychological harm.

During the past year the emphasis of Pearlin's work has shifted from identifying the types of events that arouse distress to an analysis of the processes leading to such effects. He is less concerned now with learning which events are likely to produce deleterious effects than with learning how these effects come about. A commonly accepted interpretation holds that life events create distress by upsetting a normal homeostasis, thus leaving the organism vulnerable to physical and psychological harm until a new equilibrium is established. His results, however, indicate that the manner in which life events come to adversely affect emotional functioning is different and more complex.

Specifically, he finds that where events are related to emotional distress, such as depression, a large part of the effect is indirect. This can be illustrated by his analysis of involuntary job disruptions and their consequences for depression. The following steps lead from

these undesired events to depression: first, job loss has a negative effect on income; next, reduced income produces a heightened economic strain, i.e., an intensified struggle to provide the material necessities of life; reduced income and increased strain, in turn, adversely affect self-concepts-- the lowering of self-esteem and the lessening of a sense of mastery; these contingencies then lead to depression.

The longitudinal character of the data makes it possible to establish a clear temporal order among many of the variables under analysis; Pearlin is able to observe how changes in one variable are related to changes in another variable, something that cannot be done with cross-sectional data. Moreover, because all the measures have been developed through maximum likelihood confirmatory factor analysis, they have a high degree of reliability. Both the longitudinal nature of the study and the rigor of the measures permit greater confidence in the results of these analyses than would otherwise be the case.

Pearlin has also begun to examine the role of coping responses and social supports as elements that moderate the processes that link life events and depression. Preliminary analyses indicate that social supports are most useful in blunting the distressful consequences of crises events. If crises events become converted into chronic life problems, however, social networks are less supportive. For example, if one loses one's job, then one's family and friends may provide help and encouragement; but this support may dissipate if the job loss turns into continuing economic deprivation. Supports are typically not sustained in facing chronic problems. It is at this point that people have to rely more on their own coping repertoires. Thus preliminary evidence indicates that social supports and coping have somewhat specialized functions in buffering the distressful consequences of life circumstances, with the former being especially efficacious in blunting the impact of undesired life events, and the latter in vitiating the effects of more enduring problems.

Annual Report for 1980

Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

Paul D. MacLean, M.D., Chief

Recently, there was a national conference focusing on the human family and conditions that threaten the unity, stability, and health of families in modern times. Apropos of this matter, it would be timely in the present summary of this year's project reports to call attention to aspects of the work that are relevant to the evolution of the family and that ordinarily would not be considered in a popular forum.

As an antidote for pessimistic views regarding the future of the human family, it is worthwhile to keep in mind that the "family," as a biological institution, has survived more than 180 million years, having had its origins in late Triassic times. The history of the evolution of mammals is, to a large extent, the history of the evolution of the family. In the evolution from reptiles to mammals the origination of nursing and parental care were two of the most outstanding developments. The forebears of mammals were the mammal-like reptiles which, long before the dinosaurs, populated the world in profuse numbers. Today their remains are found on every continent.

As yet the fossil record has not revealed whether the advanced mammal-like reptiles laid eggs or were attended by young. In the evolution of mammals, vocalization and hearing became of the utmost importance for parent-offspring communication and maintaining affiliation. Could the mammal-like reptiles hear and vocalize, or were they hard-of-hearing and mute like most existing lizards? There is paleobiological evidence that the mammal-like reptiles could hear, but two small bones of the jaw joint were far from being transformed into the malleus and incus typical of the mammalian ear. Two Lab projects are relevant to the evolution of vocal communication among mammals. One study involves an attempt to identify cerebral structures accounting for the "isolation peep." In an evolutionary sense, the isolation peep is probably the oldest and most basic of mammalian vocalizations, serving to maintain maternal-offspring contact, as well as contact with other members of the same species. In squirrel monkeys it has been found that structures in the midbrain tegmentum and periaqueductal gray are implicated in the production of isolation peeps, while the periventricular gray appears to influence the quality of the peeps. In the other study (conducted in the Unit for Research on Behavioral Systems [URBS]), a most interesting discovery has been made regarding the vocalization of rats. It has been found that in social situations rats may adopt individual frequency bands of ultrasound for communicating with one another and that "conversations" may continue

2

for as long as twenty minutes. The purpose of this study is to learn whether or not in an environment requiring cooperative behavior, rats will use other than their usual vocalizations to communicate with one another.

Unfortunately, there are no existing endocranial casts of the most advanced, carnivorous, mammal-like reptiles for providing information about the morphology of the pre-mammalian brain. Research on this question has been undertaken in our Paleoneurology Wing. Some external findings may be relevant to the development of endothermy ("warm blooded" state) which represents a major step in the evolution of mammals. In the most advanced mammal-like reptiles there was a disappearance of the "third-eye" (parietal eye) which has connections with the pineal body. In a parallel project it has been shown in lizards (ectothermic animals) that not only extirpation of the parietal eye, but also the pineal body, results in a two-degree rise of the ecritic temperature. Hence it is possible that the above-mentioned disappearance of the third eye may reflect a change towards an endothermic state. It is evident how relevant and important such observations are, not only in regard to understanding evolutionary changes in reproductive physiology and the necessary bodily warmth for maternal-offspring relationships, but also indeed to all biological functions in which thermoregulation plays an essential role.

The evolution of the family proceeds hand-in-hand with marked evolutionary changes of the forebrain. Essentially, there is a hierarchy of three basic evolutionary formations of the forebrain that are anatomically and biochemically distinctive. The first (the so-called striatal complex) reflects a reptilian ancestry; the second, corresponding to the limbic system, represents an inheritance from early mammals; while the third and largest formation can be characterized as neomammalian. It was previously shown in this Laboratory that in neopallietomized rodents the striatal and limbic formations are sufficient to give expression to most forms of species-typical behavior, including breeding and maternal care. Calling to mind the absence of such behavior in reptiles, play-behavior and fully integrated maternal care failed to develop in animals sustaining the additional loss of the midline limbic cortex. One of this year's projects has brought to light a finding of great interest in connection with the physiology of reproduction. The finding pertains to the pallidal part of the striatal complex, as well as to the substantia nigra, which are known to have the highest levels of iron of any structures in the brain. It has been shown in rats that the levels of iron show a predictable fluctuation during the estrous cycle, and in addition that a peak level is reached during pregnancy. Parallel changes occur in the levels of iron in the liver and serum. In male rats the pallidal and nigral content of iron is about 1.5 times less than in females. According to one scientific source, "iron deficiency is a leading national problem in the United States."

In URBS (see above) several new findings have come to light in the long-term studies on how the design of an environment may ameliorate the

untoward effects of crowding in populations of mice and of rats. In the case of mice, improved habitat design had some positive results, but did not prevent intruders from entering nests and having a fatal effect on maternal care of the young. In the case of rats, the provided opportunities for cooperative behavior appeared to have two striking effects: (1) there was a reduction in the intensity of fighting and (2) there developed a kind of reproductive control, as demonstrated by occasional maternal destruction of pups or the maternal killing of juveniles by a bite through the back of the neck. The last mentioned observation recalls the primitive human practice of infanticide and sacrifice.

The late evolutionary history of mammals is characterized by the progressive amount of attention and care that is given to the young. Mention was made earlier of the importance of vocal communication in maintaining parent-offspring contact. Separation of offspring from the mother is calamitous. We can discern in this situation the evolutionary roots of unity of the family, unity of the clan, unity of larger societies, as well as the intensity of feelings attending separation. A parental concern for the young eventually generalizes to other members of the species, a psychological development that amounts to an evolution of a sense of responsibility and of what we call conscience. In nature's record of the evolution of the family we can see as plain as day a reason for being, an ethic to live by.



Annual Report of the Laboratory of Cerebral Metabolism, NIMH

October 1, 1979 through September 30, 1980

The Laboratory of Cerebral Metabolism continues to operate as two sections, each with an independent research program on some aspects of the biochemistry of the nervous system. Because of some similarities in their physical needs, they share common facilities and equipment and occasionally draw upon special expertise peculiar to each section. The Laboratory does, however, suffer from insufficient space and positions which pose serious restrictions on its research activities, but despite these deficiencies, its research program, as judged from its recognition and respect throughout the world, has been productive.

Section on Developmental Neurochemistry

Louis Sokoloff, Chief

Most of the efforts of this Section during the past year have been on the extension and completion of applications of its recently developed [^{14} C]deoxyglucose method in neurophysiological, neuroanatomical, and neuropathological areas. Drs. C. Kennedy, S. Suda, and M. Ito have continued the collaborative studies with Dr. M. Mishkin and his colleagues in the Laboratory of Neuropsychology on the higher visual system of the monkey. These are long complicated studies on animals that have been neurosurgically prepared and trained, and progress is accordingly slow but definite. The progress is impeded only by available computer image-processing equipment and personnel needed to carry out the extensive analyses on the enormous amount of data accumulated. Studies have been initiated in collaboration with Drs. C. Gillin and M. Mishkin on the effects of natural sleep on local cerebral glucose utilization in the monkey. These studies are progressing satisfactorily. Two monkeys have been studied during sleep and two during control waking period. The results have not been fully analyzed, but it is already clear that there is widespread depression of glucose utilization throughout the CNS during sleep with some regions, such as the ventral tegmental nucleus, with increased glucose utilization. These studies offer the possibility of new insights in the nature of sleep mechanisms.

Dr. Kadekaro has completed and published her studies on the central pathways in the control of autonomic functions, such as the gastrosecretory response to hypoglycemia. She discovered 5 regions of the brain, 3 in the pathway of the vagal innervation of the stomach, which respond with increased activity to decreasing plasma glucose concentration. She has recently extended her studies to the mechanism of the hypertension in a genetic strain of rats with spontaneous hypertension and is hoping to elucidate the central neural mechanisms responsible for the hypertension.

Drs. McCulloch and H.E. Savaki have completed their studies on the effects of the dopamine agonist, apomorphine, on local cerebral glucose utilization. In addition to identifying regions of the brain that respond to activation of dopaminergic synapses, they discovered the existence of a neural pathway from the retina to the superficial layer of the superior colliculus which contains dopaminergic synapses in the retina. They have also found, in collaboration

with Dr. Agu Pert of the Adult Psychiatry Branch, that the supersensitivity of dopaminergic synapses that occurs with chronic neuroleptic therapy, has metabolic correlates which can be visualized and localized by the deoxyglucose method.

Dr. Miyaoka has completed his studies on the relationship between intensity of visual stimulation of the retina and local glucose utilization in the visual pathways. These studies have demonstrated that the metabolic activation in the projection areas of the retina is directly proportional to the logarithm of the intensity of stimulation and, therefore, follows the Weber-Fechner law.

Dr. Yarowsky has demonstrated in the superior cervical ganglion that local glucose utilization is correlated with the local electrical activity. The rate of glucose utilization is directly proportional to the frequency of electrical spike activity. Studies in collaboration with Dr. H. Gainer's laboratory in NICHD have provided convincing evidence that the stimulation of glucose utilization by increased functional and/or electrical activity is mediated by activation of the K^+, Na^+ -ATPase, an enzyme involved in ion transport and maintenance and restoration of the membrane potential.

Dr. C. Smith has completed her studies of normal aging in the rat. She has found selective decreases in glucose utilization in regions of the brain with aging. The neuropile in the cerebral cortex was particularly affected as was the caudate-putamen, and the latter also exhibited reduced responsivity with age to dopamine-agonists, suggesting loss of dopamine receptors, a possible explanation for senile Parkinsonism. She also found that the entire primary visual and auditory pathways are depressed with aging, suggesting that loss of function in peripheral sensory organs may seriously alter brain function in old age.

Drs. S. Suda, F. Schuier, and F. Orzi have been working to extend the applicability of the deoxyglucose method to pathological states. They have determined the "lumped constants", required by the method, in hypoglycemia and hyperglycemia, and the method will soon be applicable to metabolic states with abnormal glucose levels in the blood.

Dr. C. Smith has almost completed development of the method for the measurement of local rates of cerebral protein synthesis. In fact, the method is already usable, and in collaboration with Dr. B. Agranoff has found it to be very useful to study regeneration in the central nervous system.

Mr. C. Goochee has completed the development of the computerized image-processing programs for color-coding of the deoxyglucose autoradiographs. This technique has greatly extended the sensitivity and usefulness of the method, and it is now being copied by laboratories throughout the world. Mr. Goochee is currently working to improve the technique to allow complete computerized reconstructions of the entire brain encoded in color for the rates of local cerebral glucose utilization.

In addition to work on energy metabolism and protein synthesis in vivo, the Section has maintained an active research program in enzymology. Drs. E. Kaufman and T. Nelson are working on the origins and disposition of

γ -hydroxybutyric acid, a compound present in brain in minute amounts, but one that has profound effects on behavior and electrical activity. They have almost completed a method to analyze quantitatively the levels of this compound in tissues and will use it to study different conditions of brain function that might be related to alterations of the levels of this compound in brain. They have also isolated and purified an enzyme that may function in both the synthetic and degradative pathways of this compound and found that the enzyme is one that may also be related to ascorbic acid synthesis. Recently they have completed detailed analyses of the kinetic behavior of this enzyme.

Section on Myelin Chemistry

Marian W. Kies, Chief

The Section on Myelin Chemistry has two major research interests: 1) The structure and function of myelin basic protein (BP); and 2) The molecular basis of autoimmunity. Myelin is a highly specialized membrane in the nervous system essential for the maintenance of normal nerve function. The role of BP in normal function is poorly understood but its ability to induce a destructive immune reaction in certain diseases of the central nervous system (CNS) is well-known.

The interactions of BP with other lipids and proteins in myelin (whether normal or abnormal) depend to a large extent on its primary structure and conformation. During the past year Dr. Martenson has investigated the structure and conformation of BP from three points of view: 1) cleavage of rabbit BP by pepsin to obtain specific fragments for biochemical and immunologic studies; 2) theoretical analysis of the secondary structure of BP based on known physical characteristics of its constituent amino acids; and 3) studies on the nature of the interactions between BP and lipid molecules in the myelin membrane. The two latter approaches should contribute to a better understanding of how BP stabilizes the myelin lamellar structure and why the protein is a powerful autoantigen.

Mrs. Deibler and Mrs. Bacon have assisted in the isolation and characterization of BP-fragments obtained by peptic cleavage of guinea pig and bovine basic proteins. Mrs. Deibler has also prepared and characterized four V-8 protease fragments -- residues 89-119, 89-127, 120-169 and 128-169. When carboxypeptidase Y was used for C-terminal analysis of the peptides, it was found to be contaminated with an endopeptidase which is particularly active on BP as substrate. Although the contaminating endopeptidase limited the usefulness of CPY for C-terminal analysis, Mrs. Deibler completed a study of the susceptibility of BP to the endopeptidase and identified many of its cleavage sites. She also demonstrated that this cleavage of BP was not inhibited by pepstatin A as had been reported for cleavage of other proteins.

Drs. Driscoll and Richert have continued to investigate mechanisms of induction and suppression of experimental allergic encephalomyelitis (EAE). In attempting to dissect the various events involved in pathogenesis of EAE they examined the effect of in vitro culture with antigen on the ability of cells to transfer EAE to naive recipients.

Sensitized peritoneal exudate cells (PEC) of Strain 13 guinea pigs respond to antigen in vitro by marked proliferation and significantly enhanced ability to transfer EAE. Lymph node cells (LNC), on the other hand, neither proliferate nor exhibit enhanced transfer after culture with BP alone. However, if an additional "signal" is provided by simultaneous exposure of Strain 13 LNC to allogeneic (Strain 2) PEC and to BP, the LNC become capable of enhanced transfer. Thus two different cell populations display distinctly different responses to BP in culture, suggesting more than one subpopulation of BP-sensitive cells. Our current hypothesis is that one population (helper cells?) proliferates in response to antigen, and "turns on" another population (effector cells?) capable of initiating lesions in the recipient. LNC may be deficient in these helper cells although still containing effector cells capable of enhanced transfer after exposure to antigen and allogeneic stimulator cells. Guinea pig spleen cells (Spl C) have not transferred EAE under any circumstances we have examined.

Enhanced transfer is also demonstrable in Lewis rats. In this species the evidence for multiple cell populations is somewhat different. Both Spl C and LNC respond to BP in vitro (with no apparent requirement for a second "signal"). In both cases transfer is enhanced by incubation with BP, but transfer with Spl C is also enhanced after incubation with concanavalin A (Con A) in the absence of antigen. By analogy with other immune systems, both spleen and lymph nodes contain antigen-specific helper cells but the "helper function" of Con A may be mediated by a non-antigen-specific subset of T-cells found only in the spleen.

Fragments of BP prepared by other members of the Section are also being investigated in all of these experimental systems for their ability to replace whole BP for either in vivo sensitization or in vitro stimulation and enhancement. One important observation is that the effector function (disease induction) and the helper function (enhancement of transfer) can be partially dissociated: while the ability to induce EAE effector cells in guinea pigs is highly localized to one small region of the BP molecule (residues 113-121), the ability to stimulate a proliferative response in vitro and to enhance transfer of EAE with sensitized PEC is shared by more than one region of the molecule; e.g., fragment 1-88 cannot induce EAE but it can stimulate proliferation of BP-sensitized cells and enhance their ability to transfer EAE. On the other hand, fragment 89-169 possesses the total capability of the BP molecule -- the ability to induce EAE, stimulate proliferation in vitro and enhance transfer. This last observation regarding differences in the reactivity of different regions of the BP molecule should greatly facilitate our attempts to demonstrate various subsets of T-cells involved in pathogenesis of EAE.

Annual Report of the

Laboratory of General and Comparative Biochemistry

National Institute of Mental Health

Giulio L. Cantoni, Chief

The research program of the laboratory was reviewed at the October meeting of the Scientific Counselors. After hearing presentations from many members of the laboratory, the counselors' written comments about the research program of the Laboratory of General and Comparative Biochemistry were very favorable and greatly encouraging to us all. The principal obstacle to our program continues to be shortage of budgeted laboratory positions, a shortage made more critical by the recent hiring freeze. There are good reasons to be worried about our ability to continue productive research efforts in the face of budgetary restrictions.

Dr. Cantoni in collaboration with Drs. P. Chiang, Y. S. Im, A. Guranowski, and G. Kunz have continued to explore various aspects of the biochemistry of S-adenosylhomocysteine (AdoHcy) and AdoHcy hydrolase with special attention to their role in the control of biological methylation.

S-Adenosylhomocysteine was first identified as one of the products of the reactions that utilize S-adenosylmethionine (AdoMet) as a methyl donor by Scarano and Cantoni in 1954. Over the last twenty-five years, and especially in the last three or four years, AdoHcy has attracted increasing attention because it has become well established that AdoHcy is a competitive inhibitor of many, if not most, of the reactions in which AdoMet participates.

As is well known, AdoMet is one of the most versatile compounds in biology; it can function not only as a methyl donor in a very large number of methyltransfer reactions but also as a donor of a 3-amino-3-carboxypropyl group, of the amino group of homocysteinyl moiety, and after decarboxylation, of a propylamine side chain. In addition, it is cleaved enzymatically to generate thio-methyladenosine and homoserine lactone, and it can function in a variety of reactions as an allosteric effector. AdoHcy can inhibit competitively the great majority of these reactions, and thus it must be considered as a key compound in the control of the cellular utilization of AdoMet, mainly for biological methylations.

In eukaryotes, AdoHcy is metabolized through a single metabolic pathway catalyzed by AdoHcy hydrolase, an enzyme that was first characterized by de la Haba and Cantoni (1957). Recently AdoHcy hydrolase from beef liver has been crystallized in our laboratory by Richards *et al.* The enzyme has also been purified to homogeneity from beef liver by Palmer and Abeles, from lupin seed by Guranowski and Pawelkiewicz, and from rat brain by Shatz *et al.*

Little is known as yet about the comparative biochemistry of AdoHcy hydrolase. We have evidence that in beef liver there are two isozymes with very similar isoelectric properties. It is of interest that the purified enzyme from beef

liver, molecular weight of 240,000, is composed of four identical subunits, whereas the lupin seed enzyme, with a molecular weight of 110,000, is composed of two identical subunits. It is notable that AdoHcy hydrolase from the same tissue in different mammals, or from different tissues in the same animal, have different kinetic characteristics with respect to inhibitors.

In vertebrates, and perhaps in insects, the hydrolysis of AdoHcy provides the only source of homocysteine and of cysteine which is not an essential amino acid. Quantitatively, the formation of homocysteine and its hydrolysis are of major significance since the flow of methionine through this pathway is of the order of 300 μ moles/kg body weight/day. Adult mammals and cells in culture can grow normally with homocysteine (+ folic acid) as the sole sulfur source. However, after malignant transformation, cells in culture become unable to grow in the absence of methionine, although they have the biochemical ability needed for the synthesis of methionine from homocysteine and folic acid. A biochemical explanation for this interesting characteristic of certain types of transformed cells is not yet available, and the phenomenon deserves further study also in relation to the obvious implications for cancer chemotherapy.

With regard to the other product of AdoHcy hydrolase, adenosine, the interest in this compound has been greatly stimulated by the discovery in 1972, that an inborn error of metabolism, adenosine deaminase deficiency, is associated with severe immuno-deficiency involving both B and T lymphocytes. Intracellularly, adenosine can be formed only from AMP by 5'-nucleotidase and from AdoHcy by AdoHcy hydrolase; probably the hydrolysis of AdoHcy is the predominant pathway. It can be removed from the cell by release into the extracellular fluid, by deamination to inosine and by reutilization to AMP.

The competitive inhibition of methyltransferases by AdoHcy implies that the intracellular ratio of AdoMet/AdoHcy must be important in the regulation of biological methylation, and play a role in the determination of what we like to call the hierarchy of biological methylations.

Although AdoHcy hydrolase is not found in bacteria, AdoHcy is cleaved irreversibly to adenine and S-ribosyl-L-homocysteine by AdoHcy nucleosidase. This enzyme has been partially purified from *E. coli*, and found to catalyze also the hydrolysis of 5'-methylthioadenosine to adenine and methylthioribose. The enzyme is inactive towards AdoMet and a variety of other purine and pyrimidine nucleosides. The specific activity of AdoHcy nucleosidase is 1000 x greater than that of AdoHcy hydrolase emphasizing the need to remove AdoHcy, a potent inhibitor of reactions which utilize AdoMet as a substrate.

In view of the difference in the catabolism of AdoHcy in prokaryotes and eukaryotes it would be very interesting to characterize a specific inhibitor of the bacterial AdoHcy nucleosidase. Such an inhibitor would in all likelihood be a potent and nontoxic antibiotic.

The renewed interest in AdoHcy and its metabolism suggested to us a few years ago, that it would be useful to search for a potent and specific inhibitor of AdoHcy hydrolase. Such an inhibitor could be utilized to amplify the physiological antagonism between AdoMet and AdoHcy, and we hope that its use in a variety of biological systems would lead to a better understanding of the con-

trol of biological methylations. Our objective, therefore, was not the inhibition of a given reaction, but the desire to understand the control mechanism operating in biological methylations.

P. Chiang *et al.* screened a large number of compounds for their ability to inhibit AdoHcy hydrolase and identified among them a compound, 3-deazaadenosine (DZA), that is a very potent inhibitor of the enzyme. The inhibition is due to the fact that 3-DZA, like adenosine binds very tightly to the enzyme, but unlike adenosine is not a substrate for adenosine deaminase or adenosine kinase.

3-DZA, however, is not an ideal inhibitor because it can also be utilized by the enzyme place of adenosine to yield 3-DZA-Hcy. In different tissues, for reasons that we do not understand, administration of 3-DZA can produce either the inhibition of the hydrolase, with the resulting increase in the intracellular concentration of AdoHcy, or its utilization as a substrate with the formation of 3-DZA-Hcy. In most tissues both effects can be observed. 3-DZA, like adenosine, is readily taken up by the cell through facilitated diffusion, and is extremely specific in its mode of action. As mentioned above, 3-DZA is neither a substrate nor an inhibitor for adenosine deaminase and adenosine kinase. It is, therefore, not incorporated in the acid soluble nucleotide pool and hence is not incorporated in nucleic acid. As far as is known, when administered to a variety of cellular systems or to laboratory animals, all its effects must be explained by the inhibition of one or more methyltransferase reactions caused by the increased intracellular levels of AdoHcy or 3-DZA-Hcy or both. This interpretation is significantly strengthened by the fact that the biological and biochemical consequences of the administration of 3-DZA to cellular systems or laboratory animals are magnified by the simultaneous administration of homocysteine thiolactone. This compound serves intracellularly as a source of L-homocysteine and leads to the larger accumulation of 3-DZA-Hcy. 3-DZA can, therefore, be used as a probe of the participation of biological methylations in biological process, even though the specific biochemical target is generally not known.

In addition to a variety of biochemical effects, 3-DZA exerts many different biological effects in a number of cellular systems through biochemical mechanisms that are as yet undefined, but that, as mentioned earlier, must involve perturbation of biological methylations.

Leonard and Skeel, in collaboration with Chiang and Cantoni, showed that 3-DZA at 1 μ M level inhibits phagocytosis by resident peritoneal macrophages. Chiang *et al.* have shown that chemotaxis by rabbit neutrophils in response to a chemo-attractant tripeptide is inhibited under conditions where the binding of the tripeptide to the cellular receptors is not affected. Finally, Thompson *et al.* have shown that 3-DZA, and other inhibitors of AdoHcy hydrolase inhibit rapidly and reversibly synaptic responses between retinal neurons and myofibers in culture. The inhibitory effect caused by 3-DZA was potentiated more than six times by the addition of homocysteine thiolactone; when administered by itself had no effect. Trager found that 3-DZA has significant activity against *Plasmodium falciparum in vitro* (unpublished observation).

In a variety of tissue culture systems, 3-DZA exerts a powerful antiviral

action against broad range of RNA and DNA animal viruses. With J. Bader, we have studied in some detail the antiviral activity of 3-DZA on Rous sarcoma virus (RSV) infection in chick embryo fibroblasts.

3-DZA also inhibits growth and replication of other RNA and DNA viruses. The effectiveness of this compound, however, varies in the different biological systems. It is now becoming increasingly clear that both the pathways and the extent of the stepwise methylation of the 5'-cap formation varies significantly in different viruses and the same is true of the enzymes involved. It is generally believed, however, that the presence of a methyl group at the 7-position of the terminal guanosine residue is important and in some cases essential for efficient translation of mRNA.

It can, therefore, be suggested as a general working hypothesis that the efficacy of 3-DZA and other AdoHcy hydrolase inhibitors and/or AdoHcy analogs as antiviral agents relates to their ability to modulate or inhibit the reactions that lead to the synthesis of a fully methylated 5'-cap structure in viral mRNAs. Considerable experimental evidence supporting this hypothesis has been accumulated, but the critical demonstration of its validity is not yet available.

During the past year there have been several exciting advances in Drs. Mudd, Giovanelli, Datko and Thompson's continuing efforts to learn more about the biosynthesis in plants of the aspartate family of essential amino acids: methionine, lysine, threonine, and isoleucine. Very briefly, these advances may be summarized as follows: (a) Continued development of chemical means to interrupt the pathway selectively at specific points. The inhibitor, propargylglycine, which we have shown to be an irreversible "suicide" inhibitor of cystathionine γ -synthase is the latest addition to this array of inhibitors. (b) The demonstration that *O*-phosphohomoserine sulfhydrylase activity is separable from *O*-acetylserine sulfhydrylase activity. (c) The demonstration that *O*-phosphohomoserine sulfhydrylase activity is almost certainly catalyzed by the same enzyme that catalyzes *O*-phosphohomoserine-dependent cystathionine γ -synthase activity. (d) The demonstration that cystathionine γ -synthase activity appears to be regulated according to the availability of methionine, increasing almost three-fold above normal when methionine is limiting and decreasing to about 15% of normal when the plant is grown in the presence of excess methionine.

Particular interest attaches to the non-allosteric regulation of cystathionine γ -synthase since regulation of this type is thought to be extremely unusual in higher plants. Our detection of the phenomenon was greatly facilitated by the very favorable experimental system provided by *Lemna* and by the availability of specific inhibitors of the methionine biosynthetic pathway. It seems possible that non-allosteric regulation of other plant enzymes may occur, but may have been overlooked in less favorable experimental systems. We plan to examine the regulation of a number of other enzymes in the near future, as well as to attempt to look at the molecular mechanism of the regulation of cystathionine γ -synthase.

A related area of major concern has been to determine whether methionine indeed regulates its own biosynthesis in the intact plant. Such a determination is crucial to bridging the gap between *in vitro* effects on enzyme activities

and a correct understanding of how these effects impact upon the metabolic economy of the plant. We are continuing our attempts to answer this important question.

Finally, in the area of human metabolic disease, the past year has witnessed the completion of the epidemiologic study of heterozygotes for cystathionine β -synthase deficiency. The results provide no indication that such individuals, in spite of their proven mild homocyst(e)inemia, are at increased risk for atherosclerotic manifestations such as coronary attacks or strokes. This result indicates that large-scale attempts at dietary manipulation (now under discussion) based upon the possible link between mild homocyst(e)inemia and an atherosclerotic diathesis should be scrutinized very carefully before being undertaken.

The work of Klee and his associates has developed along three related pathways: (1) solubilization and purification of opiate receptors; (2) elucidation of the mechanisms involved in the pluralistic coupling of opiate receptors to adenylate cyclase; and (3) characterization of peptidases acting upon endogenous opioid peptides and their precursors.

Simonds, Koski, Streaty, Hjelmeland and Klee have achieved a significant breakthrough in their preparation of active opiate receptors in soluble form. Hitherto opiate receptors have always lost their binding activity in attempts to solubilize. Success was achieved because of the synthesis, by Hjelmeland, of a new type of detergent which is a zwitterionic bile salt derivative. Thus, for the first time, opiate receptors can be purified and their chemistry studied.

Knight and Klee have extensively purified a peptidase solubilized from brain membranes which they find has the ability to specifically liberate the pentapeptide opioid, enkephalin, from a variety of longer, presumably precursor, peptides. The existence of such an enzyme in brain opens avenues for the study of the physiological roles of enkephalin by manipulating its rate of synthesis.

Streaty and Klee continue to study the coupling of opiate receptors to adenylate cyclase. They have developed methods for quantitating agonist efficacy based upon the extent to which enzyme activity is inhibited at saturating opiate concentrations. Only opiates which exhibit at least 50% of the efficacy of presumed "pure" agonists, such as enkephalin, are able to trigger the homeostatic increase in adenylate cyclase activity associated with tolerance development.

Merril and collaborators continue to develop the two-dimensional electrophoresis system in the hope of utilizing this system as a sensitive tool for a known protein associated with metabolic diseases of genetic organisms to detect subtle differences in normal occurring proteins.

The initial studies in this collaborative project involved the development of a new silver stain for two-dimensional electrophoretograms that increases the threshold of detection of protein by approximately one thousand-fold. This allowed the study of proteins in very small tissue samples, in fluids such as

cerebrospinal fluid that have very low concentrations of protein, as well as trace proteins that may have considerable biological significance. The initial phase of the clinical research in this project has involved the establishment of normal electrophoretic patterns of clinically obtainable tissues and fluids, e.g. serum, urine, cerebrospinal fluid, cellular elements of the blood, and fibroblasts. The electrophoretic positions of known proteins in these patterns has been established. In some cases, such as human serum, a molecular map of two-dimensional electrophoresis has already been published. In other cases, particularly samples that utilize the unique properties of the silver stain, Merrill *et al.* are establishing their own normal standards. Because of their interest in the genetics and metabolism of the central nervous system, they have completed an analysis of two-dimensional electrophoresis of cerebrospinal fluid. They are currently investigating the effect of acute and chronic ethanol administration on cerebrospinal fluid polypeptides. They are also working collaboratively with the Image Processing Unit, NCI to develop computer programs to facilitate the analysis and comparison of two-dimensional electrophoresis images. This approach appears important in clinical research applications of two-dimensional electrophoresis to set objective criteria for abnormal position or amount of a particular protein.

Continued work with the silver stain for proteins in electrophoretograms has permitted the development of a rapid, relatively inexpensive silver stain. The original stain developed in collaboration with Dr. Switzer (NIMH) was an adaptation of an empirically developed histological stain for use on polyacrylamide gels. Once it was realized that the stain is based on the selective reduction of ionic silver to metallic silver at the protein sites, a process analogous to the silver based photographic methods, it was possible to adapt chemical techniques developed in photography to highly simplify the stain procedure. We now have a procedure which requires only three highly stable solutions and requires only 30 minutes. The original stain required three hours.

The permanent members of the staff have participated in a number of national and international meetings (listed below) and lectured at various universities (not listed):

Dr. Cantoni - International Conference on Biological Implications of Protein-Nucleic Acid Interactions, Poznań, Poland.

Dr. Chiang - American Society of Biological Chemist Meeting, New Orleans, LA.

Dr. Klee - Gordon Conference, Plymouth, NH; and the 4th International Conference on Cyclic Nucleotides, Brussels, Belgium

Dr. Datko - American Society of Plant Physiologists, Pullman, WA.

Dr. Thompson - American Society of Plant Physiologists, Pullman, WA.

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY
National Institute of Mental Health
October 1, 1979 to September 30, 1980
Ichiji Tasaki, M.D., Ph.D., Chief

The work currently being done in the Laboratory of Neurobiology is aimed at seeking to understand the mechanism of nerve conduction from a macromolecular viewpoint. The research includes both electrophysiological and biochemical research projects.

(A) The electrophysiological projects have included: (i) a study of the involvement of the cytoskeletal system in producing a birefringent signal associated with an action potential; (ii) a study of the mechanical changes associated with the production of action potentials in squid and in crab nerves; (iii) a study of the excitation of squid axons internally and externally exposed to single-salt solutions.

(B) The biochemical projects have included: (i) the purification of a ^3H -saxitoxin binding protein; (ii) a study of membrane-associated cytoskeletal proteins in squid giant axons and their role in nerve excitation; (iii) a study of membrane-associated proteins in neuro-secretory granules isolated from the pituitary gland; (iv) a study of developmental aspects of excitable sites in cultured muscle cells.

A. Electrophysiological Studies

(1) The birefringent signal associated with an action potential from a squid giant axon was studied as follows. Axons were injected with a very concentrated solution of a hydrophilic dye whose absorption maximum corresponded to the wavelength of the light used to illuminate the axon. Under these conditions, only the light passing through the edge of the axon, i.e. the layer directly under the membrane could reach the detector. This manipulation resulted in a much larger birefringent signal than that obtained from an intact axon. In addition, whereas an intact axon produces a biphasic birefringent signal, these dye-treated axons produced monophasic signals.

(2) The mechanical changes associated with the production of action potentials were studied using three different methods. One of the methods used was to modulate the intensity of the light from a source to a photodetector by the movement of a small piece of platinum placed on the nerve of a crab claw. During an action potential, the intensity of the light decreased, indicating that the platinum obstructed more light as a result of a transient swelling of the nerve. The peak value of the displacement was 40-80 Å. A second method consisted in using a photonic sensor to measure the movement of a piece of platinum placed on the surface of the nerve. This sensor consists of a bundle of optical fibers for transmitting light from the source to the light-reflecting target and another bundle for carrying light from the target to the photodiode. The observed fall in light intensity during an action potential again indicates a transient swelling of the nerve. Finally, a similar conclusion could be reached by measuring the pressure change of a nerve using a piezo-

electric transducer and a bender element. All three of these techniques yielded quite consistent results, both in time course and in magnitude, indicating the occurrence of swelling of nerve fibers associated with an action potential. In addition to these studies of swelling, we have observed that a transient shortening of the length of a nerve occurs during an action potential. The magnitude of this shortening is 5-10 nm and the tension developed is 15-20 mg.

(3) The excitation of squid axons internally and externally exposed to single-salt solutions was studied as follows: Squid axons were perfused both internally and externally with a solution containing CoCl_2 or MnCl_2 only (tonicity was adjusted using 12% (v/v) glycerol). At a divalent-cation salt concentration 1-10 mM, the potential difference across the membrane was close to zero. Upon application of a constant inwardly-directed current through the membrane, periodic variations of the voltage across the membrane were observed. These periodic variations of the voltage were very similar to repetitively-fired action potentials. They appeared even in the virtual absence of the concentration gradient across the membrane. The amplitude of these responses was commonly found to be 100 mV. The periodic responses obtained were accompanied by changes in the membrane conductance. The anions tested as cobalt salt included fluoride, chloride, acetate, citrate, and gluconate. There was no qualitative difference in the electrophysiological responses obtained with these anions. Nickel chloride and barium chloride were also able to elicit the periodic responses. The internal and external salt concentration could be varied independently without losing excitability. When the MnCl_2 concentration in the external medium was reduced from 10 mM to 2 mM, the duration of the response obtained by passing an inward current was prolonged. Reducing the MnCl_2 concentration of the internal medium from 5 mM to 1 mM shortened the duration of the response and enhanced its amplitude. An effect similar to that of reducing the internal salt concentration could be obtained by increasing the inward current. Tetrodotoxin (2 μM) added to the external medium did not suppress the excitability. This simplest ionic condition achieved may help advance the physico-chemical theory of nerve excitation.

B. Biochemical Studies

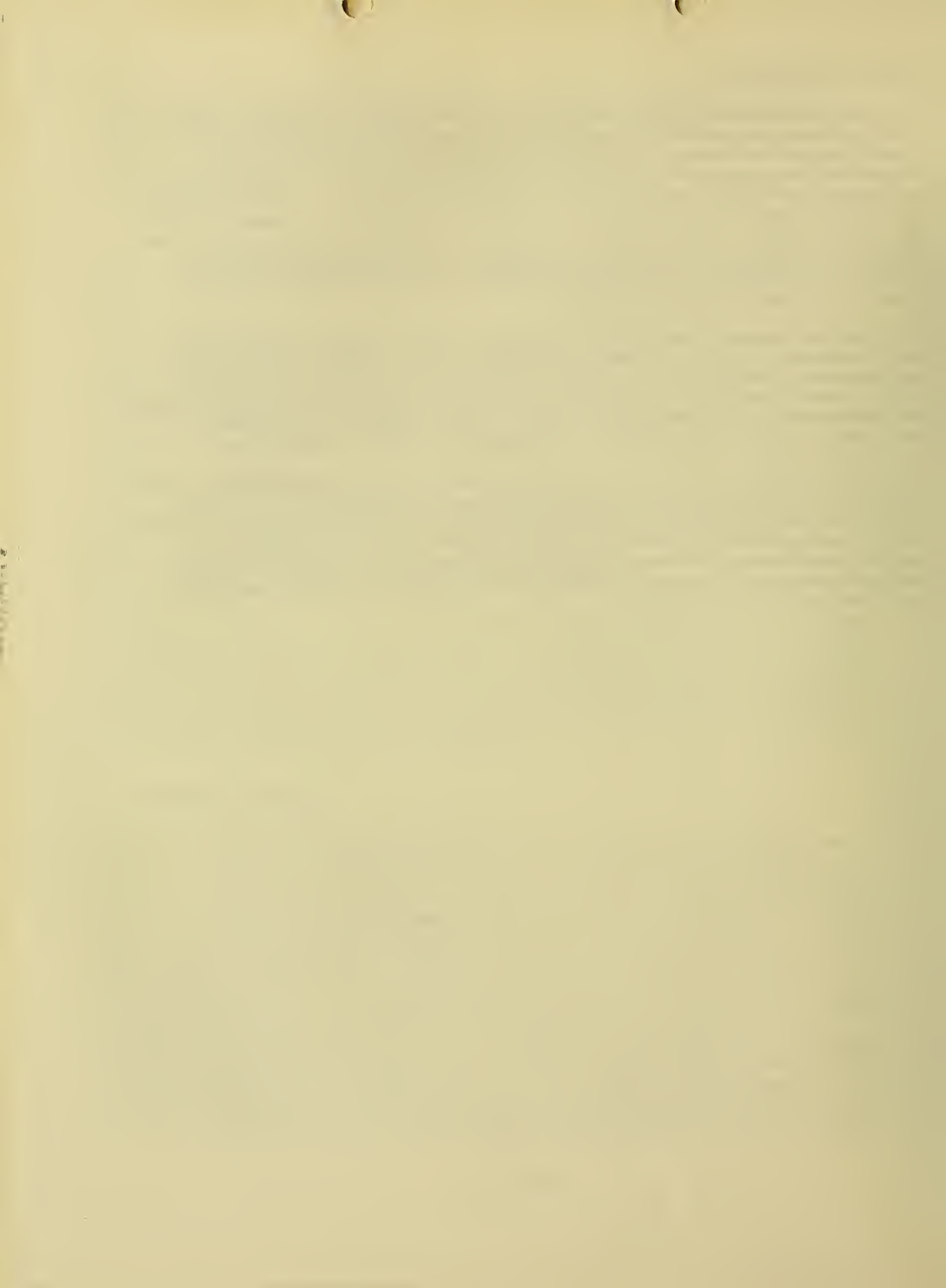
(1) The membrane-associated cytoskeletal proteins in squid giant axons were studied as follows: Cytoskeletal proteins (e.g., tubulin, actin, and neuro-filament proteins) in the squid giant axon are separable into KF soluble and insoluble forms. The KF insoluble cytoskeletal components appear to constitute the major proteins in the subaxolemmal fibrous network on the inner surface of the axon. These cytoskeletal proteins and the subaxolemmal network are both highly soluble in KI solutions. Whereas giant axons tolerate prolonged perfusions in KF solutions with no losses in excitable properties, a relatively short perfusion with KI solution completely eliminates the excitability of the axon. The loss of this excitability correlates with the simultaneous dissolution of the subaxolemmal net work of cytoskeletal proteins and the release of its proteins into the perfusate. These data support the hypothesis that cytoskeletal proteins associated with the inner surface of the axolemma are involved in the regulation of axonal excitability. This work is currently being extended by studying the excitation-dependent tyrosilation of tubulin in crab

and in squid nerves.

(2) The membrane-associated proteins in neuro-secretory granules from the pituitary were studied as follows. Granules were prepared by differential centrifugation in sucrose. The granules were then sonicated and exposed sequentially to increasingly powerful solubilizing agents. Thus, the granule membranes were first extracted with a chaotropic agent (KI), then the insoluble material was extracted with a non-ionic detergent and the remainder with an ionic detergent. These various fractions were analyzed using one- and two-dimensional polyacrylamide gel electrophoresis and have provided us with a picture of the membrane proteins from neuro-secretory granules. Further studies of these proteins are planned.

(3) The developmental aspects of excitable sites in cultured muscle cells were studied as follows: A neurotoxin from scorpion venom was purified and radioactively labelled. This toxin could be used as a probe for excitable sites since it was shown to bind to these sites. Thus, by studying the binding of this probe to cultured muscle cells at various stages of development, we were able to follow the course of development of the excitable sites.

(4) The purification of a ^3H -saxitoxin binding protein was undertaken because ^3H -saxitoxin binds to excitable sites of nerve (and muscle) and can thus be used as a probe for monitoring the purification of excitable sites. Thus far, the ^3H -saxitoxin binding protein has been successfully solubilized and a partial purification has been completed. Further work is needed to complete the purification. A number of experiments are planned using the purified protein.



Annual Report of the Laboratory of Neurochemistry
National Institute of Mental Health
October 1, 1979 - September 30, 1980

Seymour Kaufman, Chief

During the last year one of the major efforts of the Laboratory of Neurochemistry has been the continuing study of both the clinical and basic aspects of the mammalian phenylalanine hydroxylase system. In particular, the regulation of the activity or levels of the essential components of the system has been the subject of intensive investigation.

We have previously found that hepatic phenylalanine hydroxylase can be activated by phosphorylation. Furthermore, when protein phosphorylation is stimulated in vivo by administration to rats of hormones (e.g., glucagon) or drugs (e.g., theophylline), in vivo activation of phenylalanine hydroxylase can be demonstrated.

If activation by phosphorylation is a physiologically important regulatory process, a mechanism for termination of the activating process must exist. It seems most likely that the termination of activation (i.e., "de-activation") would be mediated by a dephosphorylation mechanism. We have purified extensively from rat liver extracts a phosphatase that catalyzes the dephosphorylation of phosphorylated phenylalanine hydroxylase. Just as phosphorylation leads to an increase in tetrahydrobiopterin (BH_4)-dependent hydroxylase activity, dephosphorylation is accompanied by a decrease in this activity. Neither phosphorylation nor dephosphorylation of the enzyme has any effect on hydroxylase activity in the presence of a synthetic cofactor analogue, DMPH₄. These results serve to consolidate the conclusion that regulation of phenylalanine hydroxylase by phosphorylation-dephosphorylation is a physiologically important control mechanism.

We have found, unexpectedly, that methylene tetrahydrofolate reductase (MTHFR), an enzyme with a well-established role in the biosynthesis of N₅-methyltetrahydrofolate (and hence, of methionine) has dihydropteridine reductase activity. As predicted from this finding, we have shown that MTHFR can be coupled with phenylalanine hydroxylase and can support the conversion of phenylalanine to tyrosine. It is highly likely that in the brain it can also be coupled with tyrosine hydroxylase and tryptophan hydroxylase and thus support norepinephrine and serotonin synthesis. This finding can explain why patients with a genetic deficiency of MTHFR have decreased CSF levels of neurotransmitter acid metabolites. It also seems likely that the defects in neurotransmitter synthesis can account for some of the neuropsychiatric symptoms that characterize these patients.

We have continued to explore the in vitro model for skeletal muscle hypertrophy that was developed in the Laboratory of Neurochemistry. In this model system, monolayers of chick embryonic skeletal myotubes are stretched continuously to about 10% beyond their resting length. This stimulus leads to many of the biochemical changes that are characteristic of skeletal muscles undergoing hypertrophy, including increased protein synthesis and synthesis of a muscle specific protein, myosin heavy chain.

Recently, we have found that stretching the skeletal myotubes activates the membrane Na pump ($\text{Na}^+/\text{K}^+/\text{ATPase}$). Activation of the Na pump appears to be an essential step in stretch-induced muscle growth since ouabain, a specific inhibitor of the Na pump, prevents the stretch-induced stimulation of protein synthesis. We are now investigating the connection between increased Na pump activity and muscle growth.

Dr. Neville and his colleagues have continued to study the possibility of using receptor-mediated protein transport to develop highly specific pharmacologic agents. As an example of an application of this approach, they have succeeded in modifying a normally-occurring protein, LDL (low density lipoprotein), by linking it to a sugar, monophosphopentamannose (MAN-6-P). They have shown that fibroblasts from patients with familial hypocholesterolemia, which lack the LDL receptor, and which therefore cannot take up LDL in the normal manner, can take up the LDL that has been linked with MAN-6-P, i.e., by attaching MAN-6-P to LDL a new route of entry of LDL into fibroblasts has been opened. Once the MAN-6-P-LDL has entered the fibroblast, the LDL is liberated and it expresses its physiological function, i.e., down regulation of the rate limiting step in cholesterol synthesis.

Dr. Nash and his coworkers have continued to make progress in the analysis of the system that carries out integration of a virus into the chromosome of its bacterial host. They had previously found that supercoiled circular DNA is the primary substrate for integrative recombination and that some of the products of the reaction are also supertwisted circles. Moreover, each pair of circles arising from a single recombination event is catenated, i.e., the 2 circles that are produced are joined like links of a chain. By studying recombination of DNA that is not supercoiled, they have now found that although the products of this reaction are again circles of DNA, they are not invariably catenated. These results prove that catenation is not an intrinsic feature of recombination but simply a consequence of the supertwisted nature of the normal substrate for the recombination reaction.

Annual Report of the Laboratory of Neurophysiology
National Institute of Mental Health
Edward V. Evarts, M.D., Chief
October 1, 1979 -- September 30, 1980

The projects of the Laboratory of Neurophysiology may be categorized according to two major areas of ongoing research: I) Brain Mechanisms in Movement and II) Functional Neuroanatomy. The work on movement is aimed at providing an understanding of the brain's motor control system, a system consisting of three major components (cerebral cortex, basal ganglia, and cerebellum). Research currently in progress in LNP is providing knowledge as to how the three components function in a coordinated way during movement. Such knowledge, essential to rational search for improved treatments of a number of psychomotor disorders, is being acquired by means of investigations of brain physiology and brain anatomy. Our studies of brain physiology involve microelectrode recordings of nerve cell discharge in monkeys performing learned movements. These studies have shown that cells in each of the three major components become active prior to voluntary movement. The entire cerebral cortex has massive nerve fiber connections to both the basal ganglia and the cerebellum, and these two structures in turn have powerful connections back to the motor cortex by way of the thalamus. Thus, the basal ganglia and the cerebellum receive information from the somatosensory, visual, and auditory regions of the cerebral cortex, transform this information, and then send a new pattern of signals to the motor cortex. Whereas the traditional view held that the cerebral motor cortex was at the highest level of motor integration and that the subcortical structures were at a lower level, that is, functionally closer to the spinal cord and muscle, it now appears that the situation is quite the reverse.

In addition to our studies of brain cell activity during voluntary movement in monkeys, we are devoting a major effort to collaborative research with clinicians seeking to obtain a better understanding of a variety of human illnesses in which disorders of movement constitute an important problem. Our work deals with illnesses such as tardive dyskinesia, parkinsonism, Tourette's syndrome, Huntington's disease, and other basal ganglia diseases. The goals of the work are: (1) to gain further understanding of the brain disturbances underlying the disorders, (2) to improve diagnostic and prognostic procedures in relation to motor disorders, and (3) to establish objective measures for assessing effectiveness of treatment.

In conclusion then, our work on movement involves basic research in monkeys to acquire knowledge concerning the relationships between brain areas controlling movement: experiments in monkeys allow the brain to be probed using microelectrodes and neuroanatomical techniques. In contrast, studies of the pathophysiology of movement in patients are carried out using noninvasive techniques which emphasize observations on the way in which therapeutic agents (e.g., L-dopa in Parkinson's disease) modify the underlying pathophysiology.

A second and expanding area of research in LNP is Functional Neuroanatomy. This work has involved a) tracing of neuronal fiber connections in the brain, b) development of a new in vitro autoradiography technique for localizing opiate and other peptide receptors in the brain, and c) localization of brain

sites which are selectively involved in sleep and drug-induced sedation. The fiber-tracing technique, based on transport of tritiated amino acids, has been used to show by autoradiography a variety of laminar distributions of thalamic projections to cerebral cortex. These data provide structural evidence for mechanisms of thalamocortical integration. In addition, a new method for in vitro localization of brain receptors within slices of fresh-cut brain tissue is being developed. The technique has the potential for providing a simple and general way of visualizing sites of action of a wide range of biologically active substances in any fresh brain tissue. Receptor sites for opiate derivatives and benzodiazepines are currently under investigation. A third application of the autoradiographic technique will be to visualize brain areas with special functional and metabolic characteristics. Labeled 2-deoxy glucose may reveal brain sites that have special metabolic properties during sleep states following various forms of experiences by rats.

LNP work on Functional Neuroanatomy also involves a broad, coordinated neurophysiological and neuroanatomical examination of the corticocortical connections of the sensorimotor cortex and their role in primate motor behaviors which rely upon sensory input. This work seeks to answer four major questions: first, the influence of the densest of the corticocortical inputs to the primary motor cortex (MI) is being examined by reversible cooling of the primary somatic sensory cortex (SI) and posterior parietal cortex (area 5), while recording the response of single neurons in the MI cortex to somatic sensory stimulation. Second, the responsiveness of neurons in the primary motor cortex and the supplementary motor cortex (MII), which both receive dense corticocortical inputs from somatic sensory areas of the cortex, is being directly compared in the alert, behaving monkey. Third, the precise responsiveness of neurons in area 3a, the "transition" cortex between MI (precentral cortex) and SI (postcentral cortex), to somatic sensory stimulation and the extent of its corticocortical projection to MI is being investigated. Fourth, the efferent connections of MII, especially its corticocortical and corticothalamic projections, are being examined in an effort to determine the extent to which these connections differ from those of MI.

The individual projects in progress within each of these broad areas are as follows:

I. Brain Mechanisms in Movement

1. The role of motor cortex and red nucleus in control of voluntary movement
2. Eye-head coordination in the primate: neural substrates and plasticity
3. Role of somatic sensory inputs in the cerebral control of movements
4. Motor function in patients with neuropsychiatric disorders

II. Functional Neuroanatomy

1. Corticocortical connections in the sensorimotor cortex of the monkey
2. Studies of central nervous system functional anatomy

In the summary which follows, the significance of each of these projects will be considered.

1. The Role of Motor Cortex and Red Nucleus in Control of Voluntary Movement

In thinking of central motor programs, it is useful to imagine the case of a person reaching out to touch something located an arm's length away: there is an initial large, relatively high velocity movement and then, when the hand is near its goal, small, relatively low velocity adjustments are made as the movement terminates. The initial, high velocity phase of the movement involves open-loop control, while the terminal adjustments use feedback. Open-loop control is used when the difference between the starting position and the terminal position is large and, if well-calibrated, the open-loop controller will quickly move the limb close enough to the terminal position so that the error becomes small enough for feedback to function effectively. The notion of open-loop control implies a strategy which will rapidly bridge large discrepancies from desired position at too great a velocity for feedback paths to function effectively, and rather than lumping feedforward and feedback into a single controller, it has been suggested that closed-loop and open-loop controls involve separate control strategies and thus may have separate structural embodiments.

It has been proposed that for heuristic purposes it may be useful to identify two main groups of functions for movement initiation and control: (1) the first group would deal with changes of setpoint; (2) the second group would deal with "...adaptive features of the loop at a given operating point, taking into account peripheral conditions." It seems that the first group of functions depends primarily upon the sensorimotor cortex, while the second group of control functions depends in large part upon the intermediate cerebellum and related structures. One structure which is linked to the intermediate cerebellum (via the nucleus interpositus) is the red nucleus, from which we have recorded neural activity in the past year.

These ideas concerning red nucleus and closed-loop control provide a background for our observations that red nucleus neurons (RNNs) are selectively involved in control of movement termination. We have also found that there are striking differences in the onset times of motor cortex pyramidal tract neuron (PTN) discharge and RNN discharge prior to large, high velocity movements: PTN discharge begins well in advance of the burst of muscle activity occurring with high velocity movements and peaks prior to peak muscle activity whereas RNN discharge builds up gradually during movement and peaks near movement termination. Furthermore, PTN responsiveness to limb displacement varies depending on whether the displacement occurs in the early, high velocity phase of movement (when responses are attenuated) or during precisely controlled small movements (when responses are enhanced). These changes in PTN responses depending on movement strategy are consistent with the notion that the high velocity phase is relatively open-loop, while the fine adjustments are more influenced by feedback.

In addition, we have studied activity of two other categories of antidromically identified motor cortex neurons in the same monkey and same paradigm: CRNs (cortico-rubral neurons) and PT-CRN (neurons whose axons project both

to red nucleus and to pyramidal tract). It was found that the properties of CRNs correspond to those of RNNs, while the properties of PT-CRNs are intermediate between those of CRNs and PTNs.

The significance of this project lies in its application of basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum.

2. Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

The long-term objective of this project is an understanding of the adaptive mechanisms responsible for maintaining appropriate performance levels in the oculomotor system. Three mechanisms are presently under study: (1) the regulation of the gain of the vestibulo-ocular reflex; (2) the maintenance of an appropriate match between the static and dynamic components of saccadic eye movements; and (3) a newly-discovered adaptive process that is responsible for regulating the gain of accommodation-vergence. One series of experiments on monkeys was undertaken to determine the effects of bilateral ablation of the floccular lobes of the cerebellum on these adaptive capabilities and also on the optokinetic response. A second series concerned the effects of changes in the gain of the vestibulo-ocular reflex on other systems generating smooth eye movements (pursuit and optokinetic). A third series concerned the magnitude of the compensatory eye movements associated with sudden disturbances of the head during combined eye-head tracking. Lastly, experiments were performed to determine whether gross retinal slip was necessary to induce large adaptive changes in the gain of the vestibulo-ocular reflex.

The findings in this project are of interest from a number of respects, especially since the fact that the system displays plasticity opens up new approaches to the subject of motor learning at the cellular level in the primate central nervous system. That the alignment of the two eyes, under the influence of accommodation-vergence, is subject to adaptive regulation is especially interesting since by far the most common clinical disorders of eye movements involve misalignment of the two eyes with respect to one another. The new optical techniques used in the present study were based on a systems theory approach to the regulation of eye movements rather than the "exercise approach" usual in conventional orthoptics; they may lead to new optical techniques that are of therapeutic value in the treatment of binocular misalignments in human patients. The oculomotor deficits that follow lesions of

the flocculus in the monkey demonstrate the necessity for the functional integrity of this region of the cerebellum for normal visual following and adaptive regulation in the vestibular and saccadic systems. These findings also suggest further tests that might aid localization of the pathology in human patients with classical cerebellar signs.

As our work proceeds, the adaptive capability of the oculomotor system will continue to be the main concern in these studies. Single unit studies will be employed in the search for the locus of the modifiable elements in the vestibulo-ocular reflex and will concentrate on the later stages of the disynaptic vestibular pathway. Electrophysiological techniques will also be employed in the floccular lobe of the cerebellum to determine any correlation with the adaptive mechanisms responsible for regulating the pulse-step ratio of saccadic eye movements. The newly-discovered adaptive mechanisms in the accommodation-vergence system will be examined more closely to determine time constants of acquisition and decay; studies will also be initiated in human strabismic patients to determine whether they possess such adaptive capabilities.

3. Role of Somatic Sensory Inputs in the Cerebral Control of Movements

This project is a study of the role of sensory inputs in the cerebral control of motor behavior in the primate. It uses the technique of single neuron recording in behaving monkeys to directly compare the activity of neurons in primary motor cortex (MI) during the performance of learned arm movements with the activity of neurons in the parts of the somatic sensory (SI), parietal and frontal cortex which are reciprocally connected with MI. Two questions form the focus of our investigations. First, the possible modulation of peripheral inputs to the sensorimotor cortex during motor activity is being studied by recording SI and MI response to peripheral stimuli delivered during and before active movements, during different levels of steady state motor activity related to differences in the exerted force or length of the contracting muscles and during isometric versus isotonic contractions in which the animal controls either exerted force or arm position. Second, the relation of posterior parietal and frontal cortical areas to visually guided movements is being investigated by determining whether the neuronal activity in these areas reflect information concerning the visual cue to begin movement, as well as limb position or exerted force.

Thus far, five findings have emerged.

1. Of the SI neurons found to be sensitive to passive arm displacements, 30% showed a decreased response to isotonic position pulses delivered during active movements (as compared to the responses obtained during the holding period) and in some cases responses to pulses delivered prior to onset of active movements were also decreased. Thus, MI may exert control over its own peripheral inputs via cortico-cortical projections to SI.

2. The discharge of 25% of SI neurons was related to maintained wrist position. Each of these position sensitive neurons was reciprocally related to the direction of exerted force, i.e., they were more active for one force direction and less active for the other. Furthermore, the position and force

sensitivities of most cells were congruent: cells more active with the wrist in a supinated position were also more active with supinator force and vice versa. Neurons of this type were found in three discrete zones identified as area 3a, area 2 and area 5. This indicates that neurons in cortical regions classically thought to subserve position sense are also sensitive to muscle tension and suggests that inputs to SI neurons from muscle receptors or central motor systems such as the motor cortex may play a role in perception of limb position.

3. Of the MI neurons with identified peripheral receptive fields, those classified as receiving cutaneous inputs from fingers, hand and arm were found to be concentrated in the bank of the central sulcus (MI/c) while neurons responding to non-cutaneous inputs were concentrated in more rostral regions (MI/r). MI/c neurons were characterized by a lack of sensitivity to maintained force or position and to the direction of active or passive movements while, in contrast, MI/r neurons were usually reciprocally related to the direction of active and passive movements and sensitive to maintained force and position. In addition, following the visual cue triggering active movements, the change in discharge frequency of MI/c units lagged that of MI/r units by an average of 61 msec and preceded the first change in position by an average of only 42 msec. By showing that MI/c and MI/r neurons behave quite differently during motor behavior these results confirm previous suggestions that MI consists of at least two functional motor regions.

4. A population of neurons, termed VC cells, were found to have alterations in discharge frequency which were time-locked to the occurrence of a visual cue in contrast to the onset of activity of most other neurons which was better correlated with the beginning of movement. VC activity was always specific for a visual cue triggering a particular direction of contraction. These neurons were found in two distinct groups, one in area 7 and the other in a region posterior to the genu of the arcuate sulcus. VC neurons in both groups responded with a very short latency to a visual cue, 98 msec for those in area 7 and 133 msec for the postarcuate group. Since these two cortical regions are known to be anatomically connected, these results suggest that VC neurons may be links in a pathway by which motor commands reach MI following the occurrence of a visual stimuli which requires a motor response.

5. Most of the 1612 neurons thus far studied displayed alterations in discharge frequency following a visual cue or active or passive movement; however, 314 cells (19%) showed changes in activity before either a passive displacement or visual cue or in some cases before both events. Such anticipatory activity before passive movements was seen only for neurons in MI, whereas neurons with activity changes before visual cues were more evenly distributed in both MI and SI. These results indicate that, while neurons in wide regions of MI and SI are involved in the preparation for a motor event, only MI cells become active prior to an expected sensory event.

The above results provide evidence for distinct zones of cortical neurons which are characterized by particular types of peripheral input and sensitivities to force, position and direction of active and passive movements. These zones are independent of the classical motor and sensory cortex and instead take the form of two cutaneous core zones (MI/c and area 3b-1) surrounded by

zones receiving deep inputs (area 3a, MI/r and area 2-5). In turn these regions are bounded anteriorly and posteriorly by functional zones related to visuomotor activities (postarcuate region and area 7).

These findings are of significance since further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in motor control, more information is needed concerning the characteristics of sensory input to MI, the pathway taken by this input and its role in the initiation and control of motor behavior. As an important step towards this goal, the present project examines several cortical areas through which peripheral inputs could influence motor cortex output.

4. Motor Function in Patients with Neuropsychiatric Disorders

The purposes of this project are to (i) examine the respective contributions of central motor programming and afferent information in the control of arm movements in normal, parkinsonian, and cerebellar diseased humans and (ii) to study the psychomotor performance of patients with motor disorders due to central pathology. The first set of experiments uses the techniques of recording electromyographic activity and kinematics of limb position while subjects manually match a target display with either a skilled ballistic or slow velocity movement with a handle whose displacement controls a visual display. Movement amplitude, presence or absence of visual feedback of position, and disturbances of the subject's movements are independent variables. Preliminary observations indicate that large movements are performed relatively accurately independent of manipulation of the experimental variables but that accurate performance of small movements becomes increasingly dependent on visual information and the absence of limb disturbances during movement. The second set of studies examines a variety of psychomotor variables from patients with Parkinson's disease. Tests of movement speed, reaction time, and gait abilities have provided measures that correlated with clinically determined fluctuations in drug efficacy.

In connection with the first part of this project on central and peripheral control of movement, it should be pointed out that the importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. It is the object of the present project to further examine the role of peripheral inputs in the control of limb movements. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes. Different types of limb disturbances will be imposed during the movements.

Thus far, several tentative findings have emerged:

- (1) For both parkinsonian patients and normal humans the amount of relative error increases as movement size diminishes. Positional error when velocity is first zero following movement initiation is usually less than a mean of 10-15% for the 30° movement, but can increase to 40-50% for a 3° movement. At attainment of final position, error is less than 5% for the 30° movement but often greater than 15% for the 3° movement. The presence or absence of PB does not influence positional errors when velocity is first zero but does somewhat increase error at final positioning.
- (2) In normal subjects limb perturbations at the onset of movement result in an increase of relative positional error compared to when no physical disturbances occur. This effect increases as movement size diminishes, so that, for example, error is increased to about 20% versus 10% with no disturbances for 30° movements error when velocity is first zero, and for 3° movements 60-80% versus 40-50% in the absence of perturbations. Additionally, contrary to earlier reports, the limb's final position deviates from the target position when the limb is perturbed. Thus, for large movements the final positional error can be either less (i.e., positional undershoot) or greater (i.e., positional overshoot) than the spatial value of the target; for small movements the final position is invariably greater than the target position following disturbance of the limb.
- (3) When movements of patients with Parkinson's disease are briefly opposed, the arm position tends to undershoot the target position. Since patients with Parkinson's disease perform "ballistic" movements relatively slowly, and often in a hypermetric fashion, the results obtained from this patient population must be carefully studied.

The second phase of this project deals with objective psychomotor evaluation of neuropsychiatric patients. In last year's annual report we described a computerized system for obtaining objective, quantitative measures of motor function in patients with basal ganglia disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system was used to record and subsequently summarize data concerning motor functions in 18 patients participating in clinical trials of the experimental anti-parkinsonian drug, Lisuride. Thirty age-matched normal subjects were also tested, with the intention of establishing normative data for movement variables measured on our system.

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects of normal subjects were observed.

Parkinsonian patients undergoing treatment with Lisuride were tested on a regular basis throughout the course of the buildup and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date has been permanently stored in computer files and is accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

Because of the modest cost and ease of administration, the tests that we have developed should prove to be of use in a wide variety of experimental applications that require computer recording and analyses of results.

5. Corticocortical Connections in the Sensorimotor Cortex of the Monkey

This project consists of a broad neurophysiological and neuroanatomical investigation of the corticocortical and corticofugal connections within the somatic sensorimotor cortex and their role in the generation and control of primate motor behavior. One of the central questions concerning both the synaptic and general organization of the cerebral cortex is how inputs to a cortical region from a variety of cortical and subcortical sources combine to contribute to its output. We have chosen to concentrate on the precentral motor cortex (MI), known to project directly to spinal motor neurons, and those cortical areas in close functional association with MI, the supplementary motor cortex (MII); area 3a (a transitional region between MI and the postcentral somatic sensory cortex), and the premotor cortex (area 6). Three related approaches have been adopted for this project: (1) an analysis of the organization of peripheral inputs to MI, MII, and area 3a; (2) single unit studies of cortical areas which supply inputs to MI; and (3) an analysis of MI output neurons whose targets (e.g., spinal cord, basal ganglia, cortex) have been identified.

The inputs to the precentral motor cortex (MI) as well as its intrinsic neuronal circuitry, determine the output of MI projection neurons, including those projecting to the spinal cord. Since there are a finite number of sources of afferent input to MI, as determined by neuroanatomical methods, it is a feasible objective to investigate neuronal activity within these sources during motor behavior, especially those behaviors for which MI activity is best understood. As a result of these studies we hope to gain an understanding of afferent interaction in producing motor cortex output. Ultimately, an objective of this project is to examine the activity of neurons which project to and from MI identified by antidromic axonal stimulation.

A more general objective of this project is an improved understanding of the evolution, organization, and role in voluntary behavior of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery, including MII and the premotor cortex.

Thus far, about 1300 units have been studied in four monkeys examined in this project to date; the examination of the input organization of the rostral sensorimotor cortex is nearing the completion of its initial phase and three reports of this work have been submitted for publication. Several findings and interpretations have been developed:

1. MII is virtually unresponsive to peripheral inputs, as compared with MI in the same monkey. This finding is somewhat surprising from a neuroanatomical perspective, since MII receives monosynaptic corticocortical input from most divisions of the somatic sensorimotor cortex. However, the lack of profound somatic sensory responsiveness of MII neurons supports the hypothesis that MII plays a role in centrally generated motor programs rather than movements regulated by peripheral feedback.

2. MI, which is very sensitive to peripheral inputs, can be divided into at least two regions, one rostral and the other caudal, on the basis of the broad submodality type of its peripheral inputs (e.g., skin receptors vs. muscle receptors). These results suggest that cutaneous inputs to MI, since they predominate in a discrete (caudal) part of MI, may be more important in cortical motor control than usually believed.

3. Both area 3a and MI neurons receive information concerning both the velocity and position of parts of a limb. These findings have enabled us to improve the current understanding of cerebral localization in this part of the cortex and the relationship of physiologically defined cortical regions with those defined by anatomical methods. Two of these points are most noteworthy: (a) microelectrode methods reveal that the boundary between MI and MII corresponds to the boundary between two anatomically defined parts of the agranular neocortex (termed areas 4 and 6) and (b) area 3a, the transitional field between the agranular and highly granular somatic sensorimotor cortex, appears to be, as it was originally defined (in the work of C. Vogt and O. Vogt, 1919), a discrete area characterized by a thin granular layer which receives inputs from muscle receptors concerning the velocity and, to a lesser extent, position of a limb.

Though it is often assumed in the neurological and neurobiological literature that different cortical fields relay specific information (e.g., sensory information) between each other via corticocortical connections, there is very little direct evidence which supports this conclusion. An alternative view is that corticocortical influences from a cortical region serve a number of functional roles which, in addition to information transfer (if it occurs at all), include more general influences on the activity levels or specific outputs of target cortical regions. Because of the potential significance of corticocortical connections in sensory information processing, cognition, and motor behavior of primates and other mammals, this neuronal system deserves a detailed examination.

6. Studies of Central Nervous System Functional Anatomy

The neuroanatomy research laboratory has been used this year for studies in the a) tracing of neuronal fiber connections in the brain, b) development of new in vitro autoradiography technique for localizing opiate and other peptide

receptors in the brain, and c) localization of brain sites which are selectively involved in sleep and drug-induced sedation. The fiber-tracing technique, based on transport of tritiated amino acids, has been used to show by autoradiography a variety of laminar distributions of thalamic projections to cerebral cortex. These data provide structural evidence for mechanisms of thalamocortical integration. In addition, a new method for in vitro localization of brain receptors within slices of fresh-cut brain tissue has been developed. The technique provides a simple and general way of visualizing sites of action of a wide range of biologically active substances in any fresh brain tissue. Receptor sites for opiate derivatives and benzodiazapins are currently under investigation. A third application of the autoradiographic technique will be to visualize brain areas with special functional and metabolic characteristics. Labeled 2-deoxy glucose may reveal brain sites that have special metabolic properties during sleep states following various forms of experiences by rats.

The major findings coming out of this project will be described according to the three aspects of this project:

CNS Connections

Previous work by the principal investigator utilizing the autoradiographic technique has shown a remarkable and unique projection to the most superficial layer of almost the entire neocortex originating from the ventromedial thalamic nucleus in the rat. Another widespread and hitherto unrecognized projection to the hippocampus and limbic cortices was found after placing a mixture of ^3H -leucine and ^3H -proline into the nucleus reuniens of the thalamic midline group. Results from autoradiographic cases of isotope injection into intralaminar thalamic nuclei suggest another instance of widespread neocortical distribution but, in contrast to that of the ventromedial nucleus, intralaminar projections terminate in deep cortical layers. Some other thalamic inputs are distributed to two or even three layers, and experiments have determined that multiple and spaced laminations arise from adjacent thalamic cell groups. Further analyses will also provide the topography of the thalamocortical and thalamostriatal projections. Most of this information can be corroborated from HRP case material.

A major area of thalamus whose cortical connections were hitherto incompletely described includes the ventroanterior, ventrolateral and posterior nuclei. These nuclei, previously thought to project to the motor or sensory cortex, appear to have a much wider cortical distribution. Autoradiographic and HRP experiments have demonstrated widespread cortical projection terminating in superficial as well as in deeper layers. The unique patterns of lamination, as well as a fundamental shift in views of thalamocortical connectivity provided by these data, arouse our interest in these same systems in the monkey. Such experiments could provide a bridge between the anatomical work in the rat and some ongoing and planned electrophysiological/anatomical studies concerning the primate motor cortex.

This phase of the project has resulted in development of an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. Fresh cryostat-cut brain slices are securely attached to glass slides by a process of thaw-mounting and subsequent freeze-drying. Slides are then incubated in solutions containing a labeled ligand. Excess and nonspecifically bound ligand is washed off in cold buffered rinses, and the slides are lyophilized again. The sections are fixed in hot para-formaldehyde vapors under a vacuum, then dipped in radioactive-sensitive emulsion for autoradiography. Retention of the label on the section is monitored at each step by liquid scintillation counting.

Using this method, it has been found that tissue fixation by dry, hot paraformaldehyde vapors also successfully "fixed" the tritiated ligand to the receptor. This technical breakthrough permits the use of traditional "wet" autoradiographic procedures for dipping slides into liquid nuclear track emulsion for the subsequent visualization of receptor localization. The combination of the in vitro labeling technique and "wet" autoradiography has resulted in opiate receptor localization whose resolution surpasses all previously published techniques. Through careful histological procedures we have also produced tissue of higher quality than previously attained. The same tissue is amenable to tract-tracing studies so that "double-labeling" experiments can be performed in the same animal. A paper in press describes our first efforts at surmising "opiate-ergic" tracts from the observed concordances between the autoradiographic visualization of tract terminations and receptor distributions in corresponding brain loci.

The method has applicability for all receptor types for which a drug or transmitter ligand has been identified. In addition, it can be used for cells grown in culture and on non-neural body tissues. Perhaps more exciting is the fact that it can be used on human post-mortem tissue and may open new doors into receptor-related disease states.

The CNS and Sleep

The uptake of tracer amounts of ^{14}C -labeled 2-deoxy-D-glucose (2DG) by brain tissue reflects glucose utilization of a particular region (see Project No. Z01 MH 01084-04 LNP for the method). A general lowering of brain metabolism by anesthetics occurs for most brain areas, but in this project it has been found that an anatomical system consisting of the habenula, interpenduncular nucleus and the habenulo-interpenduncular tract, shows relatively much greater labeling by ^{14}C -2DG during anesthesia. This demonstrates altered functional activity in an anatomically defined neuronal network including the interconnecting myelinated fiber tract.

Similar states of altered metabolism may occur during sleep. Current behavioral investigations done in collaboration with Dr. Martin Zatz in the Laboratory of Clinical Science in the NIMH are indicating that carbachol, a drug that mimics the action of the neurotransmitter acetylcholine, can produce profound and longterm changes in the activity and sleep-wakefulness cycle in rodents. The pharmacological and behavioral data can provide clues to the

neurochemical mediators for circadian rhythms. Alterations in physiochemical states in the brain may be measured by two techniques already described in this report--one by the 2DG method and another by the in vitro autoradiography method. The applications of these techniques must await the outcome of the behavioral experiments designed to demonstrate the role of acetylcholine in sleep cycles. Alternatively these methods might be used to show some of the CNS functions during sleep as a consequence of prior experience. Such experiments are in the planning stage.

ANNUAL REPORT OF LABORATORY OF CLINICAL PSYCHOPHARMACOLOGY
National Institute of Mental Health
October 1, 1979 to September 30, 1980

Richard Jed Wyatt, M.D., Chief

This is the last Annual Report of the Laboratory of Clinical Psychopharmacology. Before this report is published, our name will be the Adult Psychiatry Branch. This name change will reflect our expanding interests, encompassing psychopharmacology, schizophrenia, sleep, and psychiatric problems of the aged. There is perhaps a more important reason to change the Laboratory's name than simply to reflect our increased responsibilities. For some time, I have felt that psychiatry, together with its sister disciplines of psychology and sociology, has been going the way of internal medicine. During the last few decades, internal medicine has spawned a great number of subspecialties, including endocrinology, cardiology, rheumatology, and gastroenterology. While both medicine and psychiatry remain an art, their technologies are becoming too sophisticated and too diverse for a single person to fully master.

Psychiatrists, for example, are often cast as psychiatric geneticists, or psychopharmacologists, biological psychiatrists, family or group therapists, psychoanalysts. Yet, as clinicians, our work is multifaceted; we must apply knowledge of a great many disciplines to almost every patient. Furthermore, not only do we have to be generalists for our patients, but also for our research. A scientist who works on a very small problem requires a broad background to apply his findings into other areas of science. Thus, our name change from Clinical Psychopharmacology to Adult Psychiatry reflects our broader interests and perspectives.

The Adult Psychiatry Branch was the name of one of the principle clinical laboratories on the Bethesda Campus (changed to the Biological Psychiatry Branch several years ago). We certainly hope that we can maintain the tradition of excellence of the former Adult Psychiatry Branch.

Scientifically, the 1980 year has been excellent. The monoamine oxidase (MAO) story has continued to be corroborated in many institutions, and we conclude that MAO activity is decreased in some schizophrenic patients and appears to be reduced to a greater extent in either chronic paranoid or hallucinated schizophrenic patients than other schizophrenic patients. More important than subtyping, however, is the question of the physiological significance of the decrease of enzyme activity. When a decrease exists, it is relatively small. It seems unlikely to account for alterations in biogenic amines. Furthermore, even though there is genetic control of MAO activity, the possibility remains that at least part of the decrease is secondary to being schizophrenic.

During the year, however, we have published a number of studies suggesting that the decrease in MAO activity is related to physiological processes. There is, for example, a strong correlation between MAO activity and prolactin concentrations in both normal individuals and chronic schizophrenic patients. In addition, blink rate, which may be a function of central dopaminergic activity, correlates with platelet MAO activity. Finally, the same individuals who have low MAO activity in their platelets were found in the last year to have elevated phenylethylamine (an amphetamine-like substance) in their urine, both in the United States and in a population from

There also appears to be an association between decreased peripheral MAO activity and an elevation of brain norepinephrine in autopsied schizophrenic patients. (This elevation of brain norepinephrine has been found by four laboratories, including our own.) Recently, we found that brain norepinephrine concentrations could be elevated in rats with phenylethylamine. Most striking is the finding that the area (nucleus accumbens) of the brain where norepinephrine is increased is identical in both rats and humans. These findings suggest that there is a chain of events beginning with a decrease in peripheral MAO activity that then produces elevated phenylethylamine. Phenylethylamine crosses the blood-brain barrier and in a manner not yet determined increases the amount of brain norepinephrine.

Another area of major investigation during the last year has employed computerized tomography (CT) scan to investigate ventricular size and cerebral atrophy in schizophrenic patients. We found a high percentage of the chronic schizophrenic patient population at Saint Elizabeths Hospital to have increased ventricular size and/or cerebral atrophy. Most striking is that these patients have an extremely poor response to neuroleptic drugs and are inordinarily deficient on psychological testing. Furthermore, these patients seem to have elevated blood serotonin concentrations. Again, as with the MAO, it remains unclear whether these alterations are secondary to being schizophrenic or whether they are related to schizophrenia's causes. Future investigations should help resolve these issues.

This was the first full year for research on aging within our Laboratory, and this summer we will begin our first major clinical trial in patients with Alzheimer's disease. In that trial, we will attempt to decrease the quantities of blood aluminum. (Some investigators have suggested that elevated aluminum may be related to the brain deterioration.) The trial will take place in outpatients who will be brought to our research setting for periodic monitoring of their course of illness.

The major progress in our research program on aging, however, has been with brain grafting. It is now clear, at least in rats, that fetal substantia nigra grafts not only remain functional for at least one year after transplantation, but histologically and biochemically take on the appearance of dopamine-containing neurons, which are normally present in the substantia nigra. Three monkeys which received substantia nigra grafts in the summer of 1979 are awaiting shipment to Sweden where histology can be performed. They have been caught in a number of bureaucratic problems involving transport of the monkeys from this country to Sweden. This problem, however, should be resolved shortly and we expect the monkeys to be on their way to Sweden within the next few weeks.

A major activity of the Laboratory, which has attracted little attention over the years, has been our work with drugs of abuse and alcohol. This work was largely the result of Dr. Richard Stillman's efforts. Dr. Stillman is a scientist on the roles of NIDA but did his research in our Laboratory. He is on a leave-of-absence from NIDA for the next three years and is now carrying out his work in San Francisco. We have had the good fortune, however, that Dr. Elizabeth Parker, who works for NIAAA, is interested in the projects that Dr. Stillman initiated, and has taken on much of the programmatic responsibilities that Dr. Stillman began. She also is developing her own line of work. For example, her research may be able to explain, at least in part, some of the reasons why people drink alcohol. One of her most exciting findings, appearing in both humans and animals, is that if a memory task is given after social quantities of alcohol, memory for the task improves. The reason for this paradoxical improvement remains unclear and will be the subject of future efforts.

Our Sleep Laboratory continues to be under the direction of Dr. J. Christian Gillin, jointly with the Biological Psychiatry Branch in Bethesda. His activities, along with those of Dr. Wallace Mendelson in our Laboratory, will be reported in the annual report of the Biological Psychiatry Branch.



ANNUAL REPORT

LABORATORY OF PRECLINICAL PHARMACOLOGY-IR-MH

Erminio Costa, M.D., Chief

1979-1980

This fiscal year, as in the past years, the research activities and the cultural environment of the laboratory have been enriched by the presence of guest workers from various laboratories located in the USA or abroad. The help given to us by these ten guests fostered the progress of our research programs and increased our productivity. This help was in the form of critiques and collaborative work which generated our enthusiasm to integrate and consolidate concepts formed as a result of lines of investigations initiated many years ago. As an example of this consolidation, I would like to outline the contributions made this year to two old research projects: 1) the study of the mechanisms of action of benzodiazepines, and 2) the mode of action of adrenal opiate-like peptides.

In Fiscal Year 1973, we proposed that the benzodiazepines relieve anxiety because they maximize the function of GABAergic synapses. That, benzodiazepines exert their action via GABAergic mechanisms is now a widely accepted fact, which has received confirmatory support by work conducted in several other laboratories. Now, we know that this facilitation originates at specific sites in the GABAergic postsynaptic receptors where the benzodiazepines bind with high affinity to a specific protein that is operative in regulating GABAergic receptor function. The molecular nature of this facilitatory action is being investigated; it appears to depend on the benzodiazepine-elicited release of an inhibitory protein, GABA-modulin, which masks high affinity GABA recognition sites by an allosteric mechanism.

In 1977, we were the first to report that enkephalin-like high molecular weight peptides were present in adrenal medulla and sympathetic ganglia. Thus, enkephalin, similarly to catecholamines and acetylcholine, is stored in CNS and peripheral neurons. In adrenal medulla, these peptides are located in splanchnic axon terminals and in chromaffin cells. In a collaborative study with Dr. I. Hanbauer (Heart, Lung and Blood Inst., NIH, Bethesda, MD), we showed that following electrical stimulation of the splanchnic nerve, enkephalin-like peptides are released from adrenal medulla into the blood. We are now studying this secretory activity by collecting blood from the adrenal vein which is cannulated in free moving dogs. A physiological significance of this neurally mediated secretion is supported by these collateral findings: 1) stimulation of the splanchnic nerve in normal dogs causes a release of catecholamines and peptides and determines a voltage dependent increase of blood pressure which is abolished by pretreatment with hexamethonium; 2) stimulation of the splanchnic nerve of reserpinized dog causes an increase in peptide secretion without a concomitant epinephrine release; in this experiment, stimulation of splanchnic nerve is associated with a lowering of the blood pressure; administration of hexamethonium blocks the hypersecretion of medu-

lary peptides and inhibits the hypotension; 3) in rats, the intravenous administration of partially purified adrenal enkephalin-like peptides causes hypotension; 4) rat arteries contain receptors for opioids. Since in the chromaffin cells these peptides coexist with catecholamines and in the splanchnic nerve they coexist with acetylcholine, it is hypothesized that the peptides modify the sensitivity of receptors for catecholamines or acetylcholine by acting on specific opiate receptors. This action was provisionally termed cotransmitter action. This concept explains the operation of those synapses in which the presynaptic axons store and secrete two modulators like that between splanchnic and chromaffin cells. Since splanchnic neurons contain peptides and acetylcholine, and both are released by nerve impulses, we were prompted to verify the molecular nature of the cotransmitter action on target cells. On-going investigations appear to indicate that in primary cultures of chromaffin cells, the addition of opioids causes an inhibition of the high affinity binding and secretory action of nicotine receptor agonists. Hence, cotransmitters (neuropeptides) appear to reduce the number of receptors available to mediate the effect of the primary transmitter (amine?) coexisting in the same terminal with the neuropeptide. One could speculate that these cotransmitters modulate synaptic receptor sub- and supersensitivity.

Group on High Pressure Liquid Chromatography (Leader: James Meek, Ph.D.)

This group has attempted to identify the transmitters that are operative in the various afferents to the hippocampus, a brain structure of great importance in modulating emotions and affect. The perforant pathway, which links the cortex to the fascia dentata (a small area of the hippocampus) was thought to utilize a stimulant amino acid as the neurotransmitter. Previous indirect techniques could not distinguish whether this transmitter was glutamate or aspartate. Experiments conducted by Drs. Di Lauro and Meek revealed that in the fascia dentata the aspartate content decreases, selectively, after a specific lesion of the perforant pathway. This experiment provides strong evidence for aspartate functioning as a transmitter in this pathway. Serotonin has been implicated in many important functions, including sleep, sex, eating and emotion. Some of the serotonin actions may become operative in the serotonergic synapses of hippocampus. It is known that serotonergic neurons which project to the hippocampus are located in raphe nuclei; the molecular nature of the transmitters operative in the modulation of the raphe neurons is not known. To ascertain whether GABA is involved, Drs. Forchetti and Meek injected drugs that either mimic or antagonize this neurotransmitter directly into the raphe nuclei. Changes in 5HT turnover in hippocampal terminals of these raphe neurons were observed after local administration of GABA mimetics in the raphe or antagonists. These changes indicated that GABA tonically inhibits the firing of serotonergic innervation of the hippocampus.

Dr. Meek also developed techniques for the separation of neuropeptides by high pressure liquid chromatography. This research intends to avert the shortcomings deriving from the limited specificity of immunoassays used to study neuropeptide location and function. Experiments with peptides containing up to thirty amino acid residues have established the basic criteria by which peptides are retained on reverse phase columns. This line of investigation has provided practical information on how to optimize the solvent composition needed in nongradient chromatographic separations of neuropep-

tides.

Section on Molecular Pharmacodynamics (D.L. Cheney, Ph.D., Chief)

Because of the pivotal role played by the hippocampus in the regulation of emotion and learning, Dr. Cheney is studying the septal neuronal circuitry regulating the septal hippocampal cholinergic pathway. To facilitate this work various neuroactive compounds have been injected directly into the medial and lateral septal nuclei. Dr. Brunello has shown that kainic acid injected into the medial septum fails to change septal or hippocampal choline acetyltransferase activity. However, when kainic acid is injected into the lateral septum it causes a significant reduction in septal glutamic acid decarboxylase activity without altering choline acetyltransferase activity. Assuming that the resistance of a given neuron to kainic acid damage indicates the absence of somatic glutamate receptors, Dr. Brunello concluded that the cholinergic cells in the medial septum probably are not innervated by the glutamatergic axons projecting from the hippocampus. More likely, the cells directly regulated by this glutamatergic input coming from the hippocampus are the GABAergic interneurons of the lateral septum. To further substantiate her hypothesis, she conducted a series of experiments with rats under pentobarbital anesthesia. This anesthetic reduces the turnover rate of acetylcholine in the hippocampus. This effect of the anesthetic is nullified by bicuculline, kainic acid, and thyrotropin releasing hormone. From these results, Drs. Cheney and Brunello postulated that blockade of the GABA receptors, stimulation of glutamate receptors, and the interaction of thyroid stimulating hormone with its own receptors (densely present in the lateral septum) may have similar regulatory effects on the cholinergic septal-hippocampal pathway.

Recently, the use of choline and its derivative has been suggested as an expedient to increase brain cholinergic tone in patients with tardive dyskinesia and, thereby alleviate the symptoms of this disease. In order to evaluate whether a greater availability of choline could influence the turnover rate of acetylcholine in rat brain, Drs. Cheney, Brunello, and Tagliamonte administered controlled diets containing various amounts of choline. The other dietary constituents of the diet remained unchanged. The content of acetylcholine in any of the brain areas tested failed to increase as a result of the amount of choline in the diet; moreover, the levels of choline in the animals kept on a choline free diet failed to change following acute administration of choline. Actually, there was a forty percent decrease in the turnover rate of hippocampal acetylcholine in rats kept on a choline-rich diet. Dr. Brunello's results suggest that the steady-state concentrations of acetylcholine and its turnover are independent from the choline intake. However, since choline may act directly on acetylcholine receptors, one cannot exclude that the beneficial effects reported in tardive dyskinesia after choline administration may be due to a direct cholinomimetic action. In line with this possibility is the decrease of the turnover rate of acetylcholine in the hippocampus of rats on a high choline diet. Drs. Cheney, Brunello and Tagliamonte failed to find any difference in the turnover values of acetylcholine of rats immobilized in plastic cages or aluminum wire net during the infusion of the choline precursor labeled with stable isotopes. Dr. Brunello

reported that longer periods of immobilization failed to alter the turnover rate of acetylcholine in all brain areas studied. However, when hypothermia occurs during cold exposure there is a significant decrease in the turnover rate of acetylcholine in the hippocampus and hypothalamus. From these results, it can be inferred that the cholinergic systems of the hypothalamus and hippocampus participate in thermoregulation but they fail to participate in the alarm reaction in response to stressors.

Drs. Cheney and Murray are studying the effects of the potent psychotomimetic, phencyclidine, on the dynamics of various brain cholinergic pathways in an effort to achieve a better understanding of the neurochemical mechanism of action of this drug of abuse. The turnover rate of acetylcholine in the frontal and parietal cortex was increased 30 minutes after phencyclidine, but that of striatum or hippocampus was not. Dr. Murray is studying whether time and dose responses of phencyclidine to elicit a change in the turnover rate of acetylcholine and to induce hypermobility, ataxia and stereotyped behavior are related.

Drs. Cheney, and Revuelta, in collaboration with Dr. Michulam (Hebrew University, Jerusalem) have compared the effects of (-)-delta-8-tetrahydrocannabinol (THC), (+)-delta-8-THC and the dimethylheptyl (DMH) homologue of (-)-delta-8-THC with the action of (-)-delta-9-THC on the turnover rate of acetylcholine in various brain areas. They found that (-)-delta-8-THC-DMH, (-)-delta-9-THC and (-)-delta-8-THC all specifically reduce the turnover rate of acetylcholine in the hippocampus in a dose dependent manner. The (+)-isomer of delta-8-THC fails to change the steady state or the turnover rate of acetylcholine. They have speculated that the tetrahydrocannabinoids may activate specific high affinity binding sites which indirectly modulate the activity of the cholinergic neurons in the septal hippocampal pathway. Dr. Murray is attempting to test this hypothesis by direct labeling of the sites with ^3H -(-)-trans-delta-8-THC using a rapid filtration method to separate bound from free ligands

GABA turnover rate was measured in nucleus accumbens, globus pallidus, tuberculus olfactorius, nucleus caudatus, substantia nigra and septum, using a constant rate perfusion with C^{15} glucose and a mass fragmentographic method to measure the amount of the stable isotope incorporated into C^{13} glutamate and GABA at various times during the constant rate infusion of C^{13} glucose. This technique could not be used to measure the turnover rate of GABA in frontal cortex, parietal cortex, hippocampus, occipital cortex, temporal cortex and colliculi, because in these tissues the ^{13}C specific activity of GABA is greater than that of glutamate. This lack of a precursor/product relationship between glutamate and GABA can be attributed to the presence of a fast turning-over pool of glutamate which is labeled with glucose and which does not function as a GABA precursor. This glutamate pool could well be the pool of glutamate that functions as an excitatory transmitter, which is located in neurons that do not contain GABA. Dissection of the hippocampal areas CA_3 , CA_1 , subiculum and dentate gyrus has confirmed that also in these areas one cannot readily measure GABA turnover rate using C^{13} glucose to label the GABA pool. Using this method, Drs. Cheney and Revuelta are studying the regulation of GABAergic mechanisms by afferent pathways in those telencephalic nuclei where GABA turnover can be measured.

Drs. Guidotti, Kumakura and Saiani have prepared primary cultures of chromaffin cells of cow adrenal medulla. During four weeks of culture, the steady state content of cyclic nucleotides, catecholamines. Therefore, this is an ideal preparation to study various regulatory mechanisms including those that are operative in the transmitter mediated regulation of specific protein biosynthesis. Using this preparation, they were able to show that cyclic cAMP, the second messenger that accumulates in chromaffin cells following persistent activation of nicotinic receptors, produces an induction of tyrosine hydroxylase. This induction is mediated by translocation of cyclic AMP dependent protein kinase subunits from the cytosol to the nucleus, and, as shown by Dr. Kumakura, they phosphorylate a specific nuclear substrate (presumably an acidic nuclear protein). In addition, Dr. Kumakura and Dr. Guidotti found that protein kinase translocation requires the function of the microtubular proteins to be unchanged. Since cow chromaffin cells in culture maintain functional nicotinic receptors, Drs. Guidotti and Saiani are now trying to identify the optimal conditions by which stimulation of nicotinic receptors can bring about a persistent increase of cyclic AMP and an induction of tyrosine hydroxylase. This study, if successful, may give us a model for detailed studies of the selective processes by which the genetic code in chromaffin cells is controlled by the transmitter receptors located on cell membranes. Recent reports from our laboratory, as well as from other laboratories have indicated that opiate peptides are stored in chromaffin cells of adrenal medulla and in axon terminals of splanchnic nerves that innervate the adrenal medulla. Since chromaffin cell membranes contain opiate receptors, one wonders about the nature of the responses triggered by the opiate peptides secreted from splanchnic nerves when they activate opiate receptors of chromaffin cells. Drs. Kumakura, Saiani, and Guidotti decided to study whether a modification of acetylcholine receptors was operative and whether this modification has a physiological role.

Using the primary culture of chromaffin cells, Dr. Kumakura, Saiani and Guidotti have contributed evidence indicating that opiate peptides, analogous of those stored in the axon terminals of the splanchnic nerve, may function as neuromodulators of the catecholamine release induced by acetylcholine. When the opiate receptors located on these cell membranes are occupied by specific agonists, the number of nicotinic receptors and the amount of catecholamines released by maximal doses of nicotine are reduced. Because of the multiplicity of the molecular forms of opiate peptides in medulla, these results pose a number of questions concerning the nature of the agonist for the opiate receptor located on the membrane of chromaffin cells. The approach they took to solve this problem was to characterize medullary opiate receptors; they did not appear to be mu, delta or kappa receptors. Perhaps, these receptors are very close to the opiate receptors of the sigma type. Further characterization is now in progress. Drs. Guidotti and Gwynn are extending the study of neuropeptide primary transmitter interactions to a number of cell cultures which possess in their membranes multiple receptors, for instance muscarinic, opiate, GABA and beta-adrenergic receptors.

Opiate and GABA receptors participate in the control of prolactin release where they appear to act on mechanisms which are affected by dopamine. Drs. Grandison (Dept. of Physiology, Rutgers Medical School, Piscataway, NJ) and Guidotti found that opiate receptor agonists and antagonists can act in the hypothalamus but not in the anterior pituitary. In addition, opiate receptors that mediate the release of prolactin may have a different pharmacological specificity from those involved with analgesia and catatonia. In contrast, GABA receptor agonists act on pituitary but fail to act in the hypothalamus. They provided evidence that muscimol (a potent GABA receptor agonist) prevents or blocks stress or drug-induced prolactin release by acting on GABA receptors present in anterior pituitary. These GABA receptors have been characterized both by pharmacological and binding techniques. They have a high affinity for GABA (K_d 33 nM) and are bicuculline sensitive. The binding of GABA to human pituitary is similar to rat pituitary; since human pituitary contains also large concentrations of GABA (approximately 2 nM/ μ g protein)/GABA-mimetic drugs may control prolactin release also in humans.

The interactions between GABA recognition sites, benzodiazepine recognition sites and GABA modulin are operative in the regulation of GABA receptor function. Drs. Guidotti, Toffano, Baraldi, Mazzari and Leon have used different cell lines: neuroblastoma NB_{2A}, neuroblastoma N4TG1, C6 glioma, chromaffin cells from bovine adrenal and PC₁₂ cell lines to study these interactions. The GABA receptor of neuroblastoma NB_{2A} resembles the brain receptor because it includes ³H-GABA and ³H-diazepam binding sites and GABA-modulin. In this cell line, as in brain, diazepam (1 nM) prevents the inhibition of high affinity GABA binding by GABA-modulin; moreover, like in brain, GABA decreases the K_d for benzodiazepine binding to C6 glioma cell membranes. Occupancy of benzodiazepine receptors with diazepam elicits a release of GABA-modulin from NB_{2A} cells. Finally, preliminary experiments by Drs. Baraldi and Guidotti indicated that in these cells, muscimol and GABA increase the Cl⁻ influx and this increase is blocked by bicuculline. Diazepam facilitates the increase of Cl⁻ flux elicited by muscimol, but by itself fails to change the Cl⁻ influx.

Another approach adopted to elucidate the molecular nature of the benzodiazepine/GABA receptor complex is the isolation and purification of the various components of this functional complex. Drs. Guidotti, Massotti, Leon and Mazzari have worked on the isolation and purification of GABA-modulin. Their results indicated that GABA-modulin is present in rat brain as a protein of approximately 15,000 MW. This protein is rather unstable because it is metabolized by proteolytic enzymes which can produce a material of 400-800 MW which also inhibits ³H-GABA binding. Currently, the major effort of this program is focussed on the purification of the recognition site for GABA and benzodiazepines. From preliminary work it appears that each site is located on a specific protein. The affinity and number of GABA binding sites on soluble GABA recognition protein can be modulated by GABA-modulin. The modulation of benzodiazepine recognition sites by a structurally unknown endogenous agonist that is inactivated by pronase is now under investigation.

Section on Biochemical Pharmacology (N.H.Neff, Ph.D., Chief)

Various lines of pharmacological evidence support the presence of dopamine

receptors throughout the cardiovascular system as well as histological evidence for the presence of dopamine containing neuronal elements in kidney glomeruli. These observations prompted Drs. Neff and Lackovic to initiate a systematic investigation by gas chromatography mass spectrometry (GC-MS) of peripheral nerves for the presence of dopamine and its metabolites. Drs. Lackovic and Neff have identified dopamine and its acidic metabolites in rat, bovine and human peripheral nerves by GCMS which provides absolute identification of transmitters and their metabolites. In some species and in some nerves the concentration of dopamine and homovanillic acid exceeds the concentration of norepinephrine. Thus, dopamine is not solely a precursor for norepinephrine but probably functions as a neurotransmitter. From the distribution pattern in peripheral structures, it appears that dopamine may play an important role in cardiovascular and urogenital function. The actual functional role and anatomical characteristics of this peripheral dopaminergic neuronal system is currently under investigation.

Histochemical and biochemical studies carried out by Drs. Neff, and Hellstrom, (Dept. of Histology, UMEA Univ., Sweden), Commissiong (Dept. of Physiology, McGill University, Montreal, Canada) and Karoum (Lab. Clinical Psychopharmacology, NIMH) have revealed the existence of a major neuronal pathway projecting to the spinal cord from the locus coeruleus which contains norepinephrine as a transmitter. It was unclear, however, whether this projection innervates the cord ipsilaterally and/or contralaterally. Moreover, the function of this pathway was unexplored.

Drs. Neff, Karoum, and Commissiong studied the effects of unilateral electrolytic lesions of the locus coeruleus on the distribution and metabolism of catecholamines in the rat spinal cord. These results indicate that there are crossed and uncrossed coeruleospinal projections. Dopamine content of the cord remained unchanged after locus coeruleus lesions adding support for the presence of an independent dopaminergic system in the cord. Interestingly, unilateral lesions of the locus coeruleus significantly reduced the norepinephrine content in the ipsilateral hippocampus as compared to the contralateral or to control hippocampus but did not alter the dopamine content. Apparently, there is a separate dopaminergic neuronal system in the hippocampus that has not yet been unveiled.

The physiological role of the coeruleospinal pathway was studied by observing whether muscle rigidity in the intercollicular decerebrated rat changes with lesions of the locus coeruleus. In addition, Drs. Neff, Karoum and Commissiong observed that cyclobenzaprine, a centrally acting muscle relaxant, abolished muscle rigidity in the intercollicular decerebrated rat, but not in animals with bilateral lesions of locus coeruleus. These results suggest that the coeruleospinal projection plays a role in modulating the activity of alpha-motor neurons. Apparently, some centrally acting muscle relaxants activate these locus coeruleus projections.

There are many studies in the literature demonstrating that opiates interfere with dopaminergic neurons in the central nervous system and induce pharmacological effects similar to neuroleptic drugs. Drs. Neff, Parenti and Gentleman have shown that opiates and opioid peptides inhibit dopamine activated adenylate cyclase of rat striatum. The inhibition by opiates is stereo-

specific and is reversed by naloxone. Morphine also inhibits cyclase activation by GTP; however, it does not inhibit sodium fluoride activation of adenylate cyclase. These observations suggest that the inhibition by opiates may be due to an interaction between the opiate receptor and guanine nucleotide binding subunit of the adenylate cyclase complex rather than with the catalytic subunit. This evidence supports the view that endogenous opiates may modulate the responsiveness of receptor complexes to the activation by primary neurotransmitters.

While attempting to characterize dopamine binding sites in striatum, Drs. Neff and Kayaalp discovered that ascorbic acid inhibited the binding of agonists but not that of antagonists to dopamine recognition sites. This was surprising because ascorbic acid is routinely included in the incubation mixture at concentrations from 1 to 5 mM by most investigators. Ascorbic acid inhibits the specific binding of the dopamine receptor agonists, ADTN and apomorphine but not that of the antagonists, haloperidol and spiroperidol. Other reducing agents have a similar action. This observation is consistent with the notion that dopamine agonist and antagonist sites may be separate and distinct. Moreover, the number of agonist sites in vivo may be modulated in part by the state of oxidation of the binding sites.

Serotonin is found together with substance P in some neurons of brain. Dorsal root ganglia and the corresponding nerve roots contain high concentrations of substance P. Therefore, serotonin might be found in these structures as well. Drs. Lackovic, Parenti and Neff recently developed a sensitive HPLC method utilizing amperometric detection of 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid. Recently, Dr. Lackovic identified serotonin and its metabolites in the spinal nerve roots of man. Moreover, serotonin was found in some peripheral nerves and blood vessels as well.

The presence of binding sites for a transmitter on membranes prepared from neuronal tissues is currently accepted to indicate that receptors for the neurotransmitters are present in the tissue. Drs. Neff and Kayaalp identified muscarinic sites, which are distributed rather uniformly along the rat spinal cord; the receptor density is greater in gray matter than in white matter. Binding to white matter may be associated with glial cells. Within the gray matter, the receptor density is higher in the ventral horn than in the dorsal horn. Mid thoracic transection of the cord does not change receptor density. In contrast, treatment with the neurotoxin, 6-aminonicotinamide, which produces lesions of the cord, loss of motor control, and paralysis, reduces receptor density and the affinity of the ligand for gray matter but not for white matter.

Now there is substantial evidence that histamine might serve as a neurotransmitter in brain. Two types of histamine receptors have been identified pharmacologically and designated as H_1 and H_2 receptors; H_2 receptors are coupled to an adenylate cyclase system. Attempts to identify H_2 receptor binding sites in brain have not been entirely satisfactory most likely because of the relatively high dissociation constant of the radioligands available up to now. Compound ICIA 5165 (2-guanidino-4-(2-cyano-3-methylguanidine)-butylthiazol) is a new H_2 receptor antagonist with a low dissociation constant. Drs. Neff and Kayaalp found that radioactive ICIA 5165 binds to sites in

guinea pig brain that have characteristics of an H_2 -receptor. Drugs that interact with H_2 receptors in peripheral tissues displace the ligand from brain tissue. ICIA 5165 binding should be a useful aid for understanding the neurophysiological role of H_2 receptors.

In support of the hypothesis that histamine serves as a neurotransmitter in brain, Drs. Neff, Olinas and Parenti have initiated a study of histamine activated adenylate cyclase system of guinea pig hippocampus, a region where Dr. Kayaalp found a high density of H_2 receptor binding sites. By combining studies of binding sites together with studies of the histamine activation of the adenylate cyclase system they hope to gain insight into the mechanisms that modulate histamine receptors in brain.

Dopamine is contained in a subpopulation of retinal amacrine cells. Tyrosine hydroxylase within these cells exist in two states: an activated state in the light and a basal state in the dark. Activation is characterized by decrease of the K_m for the pteridine cofactor. Drs. Neff and Cohen took advantage of the retinal system to study the ability of neuroleptic drugs to activate dopaminergic neurons. In 1973, Drs. Zivkovic and Guidotti had shown that neuroleptic drugs cause a short term change in the K_m of striatal tyrosine hydroxylase. Dr. Cohen has shown that haloperidol, chlorpromazine, clozapine, or domperidone treatment resulted in an activation of retinal tyrosine hydroxylase in the dark. By kinetic analysis this activation was characterized by a decrease of the K_m for the pteridine cofactor. These results suggest that retinal tyrosine hydroxylase is modulated, in part, by dopamine receptor; perhaps, a neuronal feedback loop similar to that suggested for striatum is operative.

Section on Molecular Neurobiology (E. Costa, M.D., Acting Chief)

Enkephalins are neuromodulators which not only regulate pain threshold, but also participate in several other neurophysiological processes. In addition to the two pentapeptides, met⁵- and leu⁵-enkephalins, various tissues contain a number of enkephalin like peptides which cross react with met or leu-enkephalin directed antibodies. Some of these enkephalin-like peptides are located in central neurons; others are located in peripheral neurons: the latter regulate the cardiovascular and other neurovegetative functions.

Dr. Yang and her colleagues have documented the existence of enkephalin-like peptides in adrenal glands of bovine, human, dog, guinea pig and rat by radioimmunoassays; concentrations of peptides are higher in bovine, dog, and human adrenals than in guinea pig and rat adrenals. The significance of this marked species difference is not clear at this time. The biochemical characterization of these enkephalin like immunoreactive peptides has revealed that they are present in multiple molecular forms which can be subdivided into four fractions of different molecular weights ranging from 20,000 to 8,000, between 2000-3000, between 1000-2000 and below 1000.

The analysis of the low molecular weight fraction (below 1000) revealed that it is composed of several immunoreactive peptides which include met⁵- and leu⁵-enkephalins and two or three other enkephalin-like immunoreactive peptides. These low molecular weight peptides can also be detected by radio-

receptor binding assay indicating that they have a functional capability. One of these newly detected enkephalin-like peptides has been found to behave quite differently from met⁵-enkephalin in the radioreceptor binding assay. It can be readily detected by radioreceptor binding assay using etorphine as the radioactive ligand, i.e., it appears to be much more active than met⁵-enkephalin in displacing the ³H-etorphine bound to the opiate receptor. Furthermore, this newly detected enkephalin like peptide accounts for a large percentage of the opiate activity present in extracts of adrenal gland when opiate receptor binding is the method of assay. A very large proportion of the total enkephalin-like immunoreactivity generated by extracts of adrenal medulla is due to a heterogeneous group of high molecular weight forms (HMW forms). Two of the main HMW forms have been purified and estimated to have molecular weights of 2500-3000 and 8000. They show opiate activity in the guinea pig ileum myenteric plexus only after tryptic digestion, which generates several molecular forms of small molecular weight peptides including met⁵-enkephalin. These two HMW fractions lose their immunoreactivity very quickly when treated with carboxypeptidase A. Perhaps, the two high molecular weight enkephalin-like immunoreactive peptides are amino terminal extended met⁵-enkephalins. Drs. Stine and Yang showed that primary cultures of bovine chromaffin cells contain predominately these two HMW fractions. Drs. Yang and Harshing found that these HMW forms are very stable in the plasma and appear to exist in circulating blood. Therefore, it is possible that the HMW enkephalin-like peptides in chromaffin cells may be released into the circulation. This suggestion is further supported by the study on the release of enkephalin-like peptides from isolated chromaffin cells. Dr. Stine found that the cultured chromaffin cells spontaneously release HMW enkephalin-like peptides when cells are incubated in Krebs Ringer solution. This release is increased by 56 mM KCl and acetylcholine in the presence of Ca²⁺. Preliminary evidence indicates that the release of enkephalin-like peptides by acetylcholine appears to be due to a stimulation of nicotinic receptors; in fact, it is blocked by hexamethonium but not by atropine.

Drs. Hanbauer, Dr. Hexum (Dept. of Pharmacology, U. Nebraska Med. School, Omaha, Nebraska) and Dr. Yang could show a release of enkephalin-like peptides from the adrenal gland of freely moving dogs. Dogs were implanted with an indwelling catheter in the left adrenal lumbar vein to sample adrenal venous blood. Since the content of enkephalin like peptides in the plasma from the adrenal vein is higher than that in plasma from the jugular and femoral veins or the femoral artery, they inferred that adrenal glands secrete these peptides. The content of peptides present in adrenal vein can be increased by electrical stimulation of splanchnic nerve in a voltage dependent manner. Analysis with various fractionation methods of the peptides released by electrical stimulation has revealed that two low molecular weight enkephalin-like peptides are the main products that account for the increase in immunoreactivity elicited by splanchnic nerve stimulation. Because enkephalin-like peptides and acetylcholine coexist in the splanchnic nerve, the effect of opiates on the release of enkephalin-like peptides was also studied. The intravenous administration of morphine produces a long-lasting dose-dependent increase in plasma met⁵-enkephalin-like peptides. This morphine effect is reversed by pretreating the dog with naloxone and it requires the innervation of the adrenal gland.

In accordance with the view that enkephalins function as neuromodulators it is possible to assume that they function modulating brain excitability. A study of this role of enkephalins is possible using the hippocampus as a model. While attempting to locate the cell bodies of the enkephalin containing neurons present in hippocampus by using indirect methods following local injection of kainic acid, Dr. Hong found that a few days after intracerebral injections of kainic acid, the met-enkephalin content of hippocampus decreases immediately after injection and then it increases. This increase follows the recurrent convulsions elicited by the intrahippocampal injection of the neurotoxin. Hence, one can relate the convulsions with the met-enkephalin content of hippocampus, suggesting that a deficiency of this peptide may have a role in determining the increased excitability of hippocampal neurons.

In addition to the enkephalins, the brain contains a number of other polypeptides which function as neuromodulators. Neurotensin is particularly interesting in psychiatric research because its concentrations are higher in N. accumbens than in striatum and when injected intraventricularly causes a syndrome similar to that evoked by the neuroleptics. It is known that N. accumbens plays a role in the emotions and that it is regulated by an important dopaminergic input and receives projections from hippocampus and limbic cortex. Drs. Govoni and Yang have studied whether neurotensin containing neurons are modified by drugs that block or activate receptors for transmitters released by the neurons afferent to the N. accumbens. A variety of lesion experiments suggest that the neurotensin content of nucleus accumbens is probably located by intrinsic or short axon neurons. The neurotensin store of nucleus accumbens increases 16 hours after a single injection of 2 mg/kg i.p. of haloperidol; this increase persists for eight hours or longer. Repeated injections of 2 mg/kg i.p. per day of haloperidol cause a gradual and progressive increase of neurotensin. A significant increase of neurotensin content of nucleus accumbens is obtained with 0.5 mg/kg i.p. daily for two weeks. Maximal responses are obtained with 1 mg/kg per day for three weeks. The striatal neurotensin content of rats injected for two weeks with 1 mg/kg per day of haloperidol is also increased but by a lesser extent. In these rats the neurotensin content of preoptic area, hypothalamus, septum and amygdala failed to increase. These results suggest a modulation of neurotensin metabolism, synthesis or utilization directly or indirectly through dopaminergic synapses. This view is being tested by using a number of drugs structurally and pharmacologically related to haloperidol. So far, it appears that all the congeners endowed with antipsychotic activity can increase the content of neurotensin in N. accumbens.

Regulation of receptor sensitivity for specific transmitters plays a fundamental role in synaptic plasticity. In fact, it is now established beyond any doubt that receptors operate at variable efficiency levels, oscillating between the two extremes of sub and supersensitivity. Both states can be elicited by abnormal concentrations of the neurotransmitters. When these concentrations are very high, the receptors are desensitized. Dr. Chuang is studying the molecular mechanisms of receptor desensitization. In these studies, frog erythrocytes are a convenient model of adrenergic receptors in brain. Lefkowitz reported that incubation of these erythrocytes with high concentrations of isoproterenol causes in two hours a receptor desensitization. Dr. Chuang found that this desensitization is associated with internalization

of membrane bound recognition sites for beta-receptor agonists. This internalization is measured by the increase in the amounts of ^3H -dihydroalprenolol binding sites in the erythrocyte cytosol. Probably the decrease in the density of membrane bound beta-adrenergic agonist recognition sites induced by prolonged incubation with high concentrations of beta-adrenergic agonists is mediated by endocytosis. Neither the catalytic subunits of adenylate cyclase nor the GTP binding protein was found to be internalized under these conditions. Hence, the receptor desensitization involves internalization of recognition sites only. This internalization of recognition sites is temperature-dependent and appears to require metabolic energy. Dr. Chuang found that preincubation of cells with doses of concanavalin A, which induces erythrocyte agglutination inhibits the beta-receptor internalization, suggesting that a glycoprotein plays a role in causing this event. Inhibitors of transglutaminase (such as dansylcadaverine, methylamine, ethylamine, putrescine and bacitracin) reduced the extent of beta-receptor internalization in proportion to their antienzymatic activity. These results strongly suggest that the internalization of beta-receptor recognition sites may be preceded by a clustering of this protein which is mediated by the transglutaminase as reported previously for the internalization of polypeptide hormones and their receptors. Inhibition by transglutaminase blockers could represent the first possibility to modify receptor subsensitivity pharmacologically. Since receptor subsensitivity has been invoked to be operative in certain disease states, these experiments are being pursued in the hope of establishing a theoretical basis for future progress in the control of receptor subsensitivity.

Antidepressants relieve the symptoms of the disease by a mechanism which is still unknown. Perhaps, it is important for the therapeutic action that the beta-adrenergic receptors or other transmitter receptors are down regulated or desensitized. Dr. Kinnier has been studying the relationship between the down regulations of brain adrenergic beta-receptors and that of the high affinity imipramine binding sites also present in crude synaptic membrane preparations. This down regulation occurs during prolonged treatment with imipramine, suggesting that these two processes are not interrelated. Hence, the high affinity binding of imipramine does not appear to be a unit that is operative in beta-adrenergic function. Chronic treatment of rats with iprindole, a atypical tricyclic antidepressant fails to decrease the number of imipramine binding sites in the hippocampus, cortex and cerebellum, but still down regulates brain beta-dopaminergic receptors. This finding confirms that the high affinity binding protein for imipramine may not be operative in the action of other antidepressants. In order to facilitate a better understanding of the role of this protein, Drs. Chuang and Kinnier characterized the properties of high affinity imipramine binding sites using rat hippocampal membranes. This binding activity is located on a polypeptide because it is diminished by treatment of the membranes with pronase or phospholipase. Moreover, the B_{max} of the binding is decreased to one-tenth of the original value when the binding temperature is increased from 0 to 37°. The mechanism of such an inactivation is now under investigation.

Substance P is a neuromodulator located in neurons that are operative in sensory afferents to the spinal cord. Because of the importance of this modulator it is necessary to understand how its synthesis is regulated in order to design strategies for the development of new drugs. Drs. Schwartz

and Ross found that chick embryo dorsal root ganglion contains a molecularly heterogeneous pool of substance P-like peptides including a high molecular weight species which could function as a precursor in the formation of Substance P. The content of this possible precursor is regulated by treatment of the ganglia with nerve growth factor. In order to carry out pulse labeling and chase experiments to demonstrate the nature of the precursor/substance P relationship, methods for culturing dissociated neurons from chick embryo dorsal root ganglia have been developed. These cultures are also being used for studies on the mechanism of action of capsaicin, a specific neurotoxin for substance P neurons.

In C6 glioma cells, beta-adrenergic receptors are linked with adenylate cyclase; hence these cells can be used to study the molecular biology of long protein kinase stimulation following long term activation of beta-adrenergic receptors. Stimulation of these receptors with isoproterenol results in a sequence of events leading to two ultimate responses: an increase in the content of nerve growth factor and an increase in the activity of the cyclic nucleotide phosphodiesterase. The initial events mediating both of these responses are the same, a rise in cyclic AMP and an activation of the cytosolic cyclic AMP dependent protein kinase. However, the increase in nerve growth factor is an activation process that does not require protein synthesis, while the increase in phosphodiesterase is an induction process that does require protein synthesis. Dr. Schwartz is attempting to isolate and purify pro-beta-nerve growth factor in order to examine the role of protein kinase in stimulating the conversion of pro-beta-nerve growth factor to nerve growth factor. The increase in phosphodiesterase elicited by stimulation of beta-adrenergic receptors requires both RNA and protein synthesis. Dr. Onali found two forms of phosphodiesterases present in the cytosol of C6 cells and he separated these two enzyme forms by DEAE Sephacel chromatography. One form that metabolizes both cyclic AMP and cyclic GMP was activated by calcium and calmodulin. Drs. Onali and Schwartz found that the other form that utilizes only cyclic AMP as a substrate is specifically induced by beta-adrenergic stimulation of the cells. Dr. Schwartz found that the induction of phosphodiesterase requires a translocation of catalytic subunits of protein kinase into the cell nucleus. Both the translocation and PDE induction were blocked by drugs that interfere with microtubule function, such as vinblastine and colchicine, expanding a concept formulated after the work on the induction of TH in chromaffin cells, that translocation of protein kinase catalytic subunits from cytosol to the nucleus requires intact microtubular function. Studies on the phosphorylation of various fractions of nuclear proteins suggest that an acidic protein(s) is a preferential substrate for the translocated protein kinase. Cordycepin, which inhibits the stimulation of acidic protein phosphorylation also blocks phosphodiesterase induction by isoproterenol. Activation of RNA polymerase II is also required for phosphodiesterase induction, presumably because it mediates the synthesis of the mRNA for phosphodiesterase. Drugs such as actinomycin D and alpha-amanitin inhibit RNA polymerase II activity in vitro as well as prevent the phosphodiesterase induction.

Drs. Schwartz and Breakfield (Dept. of Genetics, Yale University, New Haven, Connecticut) demonstrated that cultures of human skin fibroblasts synthesize nerve growth factor and the content of nerve growth factor can be regulated by the beta-adrenergic receptors. The nerve growth factor present

in fibroblasts from dysautonomic patients has one-tenth the biological activity of fibroblasts from normal volunteers. Furthermore, the content of nerve growth factor in dysautonomic cells cannot be increased by treating the cells with beta-adrenergic agonists; such an increase can be elicited in normal fibroblasts. These results suggest that in familial dysautonomia there is a primary defect in the pro-beta-nerve growth factor molecule. Dr. Sredni (guest worker from Israel) is now isolating pro-beta-nerve growth factor from mouse submaxillary gland. Drs. Schwartz and Breakfield are also doing experiments in which the fibroblast cultures are incubated with radioactive amino acids. The extracts are immunoprecipitated with the antibody to the beta-nerve growth factor, and the precipitate is separated on gel to assess the differences between the control product and the dysautonomic product. The feasibility of measuring nerve growth factor in fibroblasts grown in amniotic fluid is now being studied. Preliminary results suggest that this approach may be a good method for prenatal diagnosis of familial dysautonomia.

Dr. Vargas is testing whether, similarly to GABA, also the receptors for other amino acid neurotransmitters are regulated by mechanisms designed to protect them from stimulation by the circulating amino acids. The receptor for glutamate which is a very important brain excitatory neurotransmitter appears to be protected by a specific inhibitor. Dr. Vargas has shown that a Ca^{2+} -dependent cysteine proteinase regulates high affinity binding for glutamate in hippocampal crude synaptic membrane preparation. The facilitation of glutamate binding elicited by the stimulation of Ca^{2+} -dependent proteinase appears to depend on the destruction of an inhibitor for the high affinity binding of glutamate. Since this regulation is more evident in cortex and hippocampus than in cerebellum, a functional polymorphism of brain glutamate receptors can be considered. The facilitation of glutamate receptor function elicited by the stimulation of the Ca^{2+} cysteine proteinase is being studied as a potential site for the action of centrally acting anticonvulsants, such as diphenylhydantoin and barbiturates.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

October 1, 1979 - September 30, 1980

The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); (4) provision of other technical services in support of the research program.

The Branch is comprised of two sections:

Section on Instrumentation and Computers provides technical support for investigators by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Section on Laboratory Animal Medicine and Care provides professional advice and assistance to intramural scientists on all aspects of animal health, medical care, and testing and surgical protocols; manages a central primate holding facility; and conducts a program of laboratory animal care.

Additional services provided by the Branch include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Branch personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Branch is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Branch, he first meets with the Branch Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether RSB will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, RSB will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Branch Chief or the Assistant to the Chief agree to accept a project, the investigator submits a standard work request form (available from RSB), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The RSB does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Branch's CAN.

SECTION ON INSTRUMENTATION AND COMPUTERS

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 288 projects, and are representative of the types of electronic instruments and systems developed by the Section:

(1) Patient Activity Monitoring System. The Section has continued to develop the NIMH Activity Monitor (PAM) and the ancillary hardware and computer software which forms the system. The major development of the past year has been the hybrid monitor. This device, developed under contract by a local thickfilm hybrid circuit company, features a memory with the capacity for 10 days of continuous monitoring at 15 minute resolution. Unfortunately, the development was plagued with problems, and to date only 35 of the contracted 50 devices have been delivered. These have proved to be somewhat unreliable because they are constructed with untested chips, which have a high infant mortality rate. On the basis of our experience, we have decided to abandon this form of the monitor, and we will not contract for additional hybrid monitors.

Since the 10 day monitor is essential to outpatient studies, the Section is now designing a new device based on a technology which uses packaged tested chips, and newly developed high density memories. It is anticipated that a device with 10 day memory and one year battery life can be produced in the size of the present 64 hour monitor. Initial production should begin late this year.

In the interim, the Section continues to produce the standard device, for new applications and to replace old monitors. More than 100 standard monitors are now in the field.

(2) Dyskinetic Mouth Movement Detector. A small, battery powered unit was developed for use in measuring, on a continuous basis, abnormal oral movements associated with tardive dyskinesia. Several accelerometer transducers

were designed for the system to record the specific dyskinetic movement the patient might manifest, such as cheek puffing or lip pursing. The transducer output signal is connected to a modified Patient Activity Monitor which records the number of abnormal movements for the time interval selected, and store the number in memory to be read out later on a computer. A calibration box allows the investigator to determine the threshold point at which a movement is recorded. Two units were built; a one-minute interval unit which will store over 4 hours of data, and a second unit with a seven and a half minute interval which can store up to 32 hours of data.

(3) Parkinson's Disease Testing Station. Work has continued on developing instrumentation to be used in recording data on patients with Parkinson's disease. One of the neurological manifestations of the disease is tremor. New tri-axial accelerometers were developed that enable the tremor amplitude and frequency to be recorded and analyzed by the computer program. In order to verify the data recorded, a calibration system was developed including a mechanical device that will produce a 5 Hz sinusoidal movement at a fixed amplitude. This device can be used at the recording site to make sure the accelerometers and program are working properly. In addition, instrumentation was developed to provide more extensive calibration data in verifying acceleration constants at various amplitudes and frequencies.

The equipment used for the reaction and movement time measurements was also improved. New touch pads, circuitry and base were constructed to provide a more reliable and accurate means of recording these parameters.

(4) Exploratory Activity Cage. An activity cage has been developed to quantify the exploratory behavior of laboratory rodents. One-third of the area of a plastic animal cage forms a dark chamber which is separated separated from the lighted two-thirds by a partition. Four sets of IR diode emitters and phototransistor detectors positioned across an opening in the partition are used to count the number of times the animal crosses the partition and to measure the length of time spent in each compartment. Potential false transitions caused by tail movements in long-tailed rodents are rejected by the emitter-detector spacing geometry and the associated circuitry. It is hoped that this apparatus will provide a rapid, simple test for pharmacological studies of drug effects on exploratory activity.

(5) Rat Sleep Scorer. A device to categorize rat sleep states by real-time analysis of bifrontal and frontal-occipital electrocortical activity and electromyographic activity has been under development for several years. The algorithm which has evolved now achieves an acceptable scoring accuracy as verified by extensive tests. The scoring hardware has been interfaced to a line printer so that a formatted, chronological sleep-state record is produced to facilitate entry into a minicomputer for secondary analysis. Design of a multichannel system with complete, stand-alone analysis capability has been initiated.

(6) Electromagnetic Field Control System. The use of small sense coils within orthogonal electromagnetic fields has become a widely used method of recording horizontal and vertical eye movements. Vision studies involving long-term single unit recording place stringent requirements on the field-generating electronics. These include high frequency operation (20-30 KHz),

both short and long-term stability in field strength and in field orthogonality, and the ability to maintain the stability of these parameters when moving metallic objects are within the fields. An electronic field coil system has been developed to operate at high frequency; it uses automatic gain and phase control to obtain the required operating stability. Independent adjustment of the two field strengths and the phase are provided.

(7) Rat Startle Response Measurement. Startle response, an important unconditioned reflex behavior, is used in studying how the nervous system is modulated by various environmental factors. A device was developed which accurately quantifies three parameters of the startle reflex: latency before response; time until peak force; and maximum peak force. The isometric force measurements are transduced by four semiconductor strain gages mounted in a double cantilever fashion. The device is both manually and microcomputer controllable, and will resolve forces from 10 to over 500 grams and times from 1 to 256 msec. Data can either be recorded manually from LED displays or by the microcomputer from a data bus for further analysis.

(8) Multichannel Programmable Stimulus Generator. This microprocessor-based instrument, currently under development, will provide a stimulus pulse output per lever press input during a period of time referred to as an epoch. The operator will be able to specify the duration of the epoch, the total number of epochs, the duration of the stimulus pulse in milliseconds, and the percent of time within a given epoch when stimulus pulses will be generated by a lever input. An additional feature will provide a listing of lever inputs per channel per epochs.

(9) Programmable Infusion Pump. This instrument, a motor driven syringe controlled by a microprocessor, can be programmed to provide pumping schedules which are various mathematical functions specified by the operator. When the appropriate input arguments are entered on a console, exponential, constant, and ramp infusion rates can be realized.

(10) Rat Activity Monitoring System: Solid-State Imaging System. The development of a new method of monitoring a rat's activity over a large area was begun. The activity cages using capacitance transducers, as described in previous annual reports, are only effective for areas up to 90 mm square. In order to monitor rats in an enclosure of 1 meter square a new technique was needed. It was felt that the activity could be monitored by a closed circuit TV system, but this was too expensive. A solid state imaging system, consisting of a special lens system in conjunction with an 8x8 array of discrete phototransistors, was designed and is being tested. An overhead light casts a shadow of the animal on a translucent floor. This image is focused by the optical system on the phototransistor array in such a way that a single square of the floor, 1/8 meter on a side, projects onto a single phototransistor. Thus, it is possible to obtain an output of the transistor which is proportional to the amount of the square which is occluded.

COMPUTERS

The Section on Instrumentation and Computers continues to support the use of the computer as a laboratory instrument. Small computers are used in the individual laboratories for on-line, real-time interaction, process control and data acquisition. The Section maintains support computers in Buildings 10 and 36. These systems provide means for program preparation, bulk storage, printing and plotting, and mathematical and statistical processing. Experimental data may be transmitted from the laboratory computers, via these systems, to the DCRT facilities for further processing. The support computers also serve to develop prototype systems and to test the feasibility of the use of a computer in specific laboratory applications. The latter capability allows an investigator, once he determines that the computer will do the job, to purchase an efficient system at minimal cost.

The Section provides software support for the individual investigators. A library of procedures has been developed that is tailored to the needs of the Intramural Program. Individual training is available for investigators with no prior experience in using or programming the computer. Computer specialists are available for consultation for all areas of computer use, programming, interfacing, real-time applications, time series analysis, data presentation, systems configuration and computer procurement. Although we do not provide an applications programming service, systems have been developed in collaboration with individual laboratories. Examples are included in the list of computer projects.

An increasing amount of time is devoted to program maintenance. Computer programs, especially in research related services, are not static and often require development, improvement or modifications. In addition to the software library and research related projects developed by the Section, much work is caused by the turnover of scientific and support personnel. Many systems developed by these persons prove useful to the laboratory. After they leave, maintenance of such systems often falls to the personnel of the Section. We have investigated the use of structured programming techniques and programming languages in an attempt to define software standards. PASCAL was chosen as a standard language. As the problem of program maintenance continues to grow, use of these techniques will be necessary to enable the Section to furnish this service without an increase in personnel.

The workload on the Section's computer facilities has reached the saturation point. A second support computer for Building 36 has been ordered. The Section has begun a system study for the possible acquisition of a new 32 bit minicomputer. Operating systems are available for these computers which support multiple users, but it is not clear that they may be implemented without adding personnel. They are particularly suitable for graphic image processing and digital signal processing. Techniques for digital signal processing are particularly applicable to neurophysiological data and such applications are continually under development by the Section. Even with the investigators working evenings and weekends, these applications take much time from program development and testing. The larger minicomputer system would provide greater precision, faster computation and multiple users. In

addition, programs not requiring real-time data acquisition or process control could be developed on a system of this type.

One of the major functions of the Section is to provide systems studies for use of the computer as a laboratory instrument. The concept of the microcomputer as an integral part of laboratory instrumentation for process control, data logging, timing and coordination of instruments has proven to be both cost effective and efficient for laboratory applications. Mini- and microcomputer based systems provide a wide spectrum of tools for research ranging from lower cost data loggers to sophisticated systems which interact with the on-going experiment. These systems have resulted in a further integration of the engineering and computer functions of the Section and have enabled us to offer a wide range of computer related services. Projects such as the Parkinson's Disease Testing Station are the result of close coordination between the scientist, the electronic engineers, and the computer scientist and illustrates a type of service not feasible a few years ago.

IMAGE PROCESSING SYSTEM

The Section on Instrumentation and Computers general purpose image processing system became operational in the spring of 1980. The system consists of a high-speed rotating drum scanner, an image array processor and display, and a PDP-11/60 computer. The drum scanner can digitize transparencies up to 10x10 inches with spatial resolution of 12.5 microns. The image array processor can simultaneously store, display, and manipulate up to three 512x512 digitized images. Images may be compared, superimposed, translated, zoomed or color coded at video rates. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using previously digitized images generated by CAT or ECAT scanners.

An interactive, menu driven, software system is being developed to provide an extensive and expandable repertoire of basic image processing and input/output functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. Two applications, however, are primary: analysis of two-dimensional electrophoresis gels and analysis of autoradiographs of brain or tissue sections.

The autoradiographs are used for measurements of glucose utilization in brain tissue using the Sokoloff [^{14}C] deoxyglucose method of glucose substitution. Analysis of the autoradiographs involves displaying the digitized image on a TV monitor and outlining areas of interest. The average optical density is then computed and automatically converted into glucose utilization. Glucose utilization of brain regions as small as 100 microns in diameter can be computed. A color coded glucose utilization map may also be produced.

Measurement of amino acid concentrations can be made using two-dimensional electrophoresis gels. The gels, which have been prepared by the appropriate stain and fixer, are photographed; or if radioisotopes are used, an autoradiograph is obtained. The film is scanned and digitized into an array of optical densities. The resultant image is then displayed and analyzed. Measurement of individual amino acid concentrations can be made by integrating

optical density within a defined boundary. A test gel may be compared with a standard gel using the image array processor to determine the presence or absence of a particular substance.

Additional examples of computer projects include:

(1) Membrane Activity of Neurosecretory Cells. This system was developed in collaboration with the Laboratory of Neurophysiology (NINCDS) to study the conductance of cell membranes that often exhibit bursting activity during clamping. The system is being upgraded to record and monitor the tissue culture neurons on-line and to allow the iontophoretic or pressure injection of neuroactive substances onto the external surfaces of the cells. The purpose is to discover the nature of the action of such substances on the properties of the cell membrane.

(2) Cell Culture Analysis. This system is designed to provide an on-line analysis of tissue culture neurons. The first phase, to study the excitatory or inhibitory post-synaptic potentials of these cells, has been completed. A unique feature of this system is the on-line control of artifacts introduced by the measurement system and the properties of tissues in culture and to control the threshold levels and amplification level as the experiment is in progress. Visual displays of amplitude, integral and latency are available, as well as averaged evoked response. In addition, on-line monitoring of post-synaptic potentials elicited by stimuli presented in pairs or in trains of pulses are available. The system also studies spontaneously occurring miniature potentials. This system will be extended to allow analysis of the cells by other techniques such as voltage clamping and the iontophoretic injection of neuroactive substances on the surface of the cell.

(3) Rat Activity Monitoring System. The system designed to measure gross movements and rearing activity of white rats was extended to differentiate between two levels of gross movements and to monitor the amount of time the animal spends in each quadrant of the cage.

(4) Membrane Channel Analysis. Acetylcholine molecules outside neural membranes open and close channels allowing the passage of Na^+ and K^+ ions. Current fluctuations resulting from single channels opening and closing have a high signal-to-noise ratio. This program catalogues and identifies the duration and amplitude of channel activity, and provides a spectral distribution of the electrical activity of the membrane.

(5) Cytofluorographic Analysis of Single Cells. The system designed for cytofluorographic cell sorting by the Computer Systems Laboratory, DCRT, is being modified for the cytofluorographic analysis of cells in immunological studies.

(6) Process Control System. A process control system was developed to allow an aperture controlling a narrow beam of light to be projected across the visual field of the retina of experimental animals. The beam may be centered on the retinal cell from which a microelectrode is recording responses. The beam may be automatically passed across this light at controlled rates and controlled vectors. The system is designed to give the investigator flexibility in stimulus presentation, freeing him for observation of the response.

(7) PAM Readout Station. An LSI-11 system with a printer/plotter and video graphics was developed and installed in Bldg. 10 to support activity monitor readouts and analyses. Software was also developed to include the new hybrid monitors and monitors with 1 hour sampling intervals. The programs now allow readout of the commercially made temperature monitors, using the same software as the activity monitors.

(8) Tremor Analysis System. One important feature of the Parkinson's Disease Testing Station is the analysis of tremor data. The development of a tri-axial accelerometer enabled computation of various parameters associated with the tremor characteristics, including dominant frequency, intensity of tremor at that frequency, and total movement in a fixed interval. An estimate of dyskinesia could also be quantified. These computations all employ spectral techniques associated with FFT algorithms. A calibration system, including hardware and software, is being developed to allow the operation and accuracy of the system to be periodically checked.

(9) Respiration Analysis in Monkeys. A system of programs was developed to analyze data involving single neural unit spike trains and respiration in monkeys. To overcome inherent hardware problems, software filtering techniques and sophisticated statistical tests were used to establish correlations between respiration and neural activity in the limbic system of these monkeys. Extensions of these software techniques have application in other problems involving correlation between digital and analog signals.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care is staffed by a veterinarian, laboratory animal technicians and animal caretakers who provide daily care and maintenance of laboratory research animals. Services provided by the Section include:

(1) Management of the Central Primate Facility. The Central Primate Facility has a capacity for approximately forty primates. At the present time, three investigators have research projects in progress. Support is provided through assistance in preparing surgical models, insertion of catheters and maintaining animals on special diets and feeding routines. Thirty-five anesthetic and/or surgical procedures were supported this past year. The Section has also developed the capability for bacteriological culturing and identification, pH and blood gases and non-invasive blood pressure monitoring.

(2) Tuberculosis Testing Program. All primates, including those located at St. Elizabeth's Hospital are tuberculosis tested every three months.

(3) Treatment of sick and post-operative animals.

(4) Assistance in animal procurement when needed.

(5) Providing day to day care and maintenance of research animals. Care and maintenance of research animals are provided seven days a week in all

areas. The supervisor of the Section provides day to day contact with personnel in all work areas and coordinates weekend and holiday schedules. Night coverage of the primate nursery continues to work very smoothly with two part-time persons covering on alternating nights. Also, these two persons administer night medications to animals on treatment.

(6) Upward Mobility Training. The program continued this past year and the person selected finished the training in March 1980. Another person will be selected in the near future to continue the program. The goal of this program is to select personnel for further training who have become proficient in their job but lack a career ladder to learn new skills and techniques for further advancement. The training period lasts for eight months, after which the trainee returns to his former duties with qualifications for advancement to the biological technician (animal) field.

(7) Animal Facilities. Upgrading of the animal holding facilities continues. New rodent and rabbit cages and a rack-size laminar flow unit were purchased for the animal rooms in Building 10. Primate cages were purchased for Building 36, completely replacing all the old cages.

Although the animal holding facilities at St. Elizabeth's Hospital and Poolesville are not part of the Section due to logistic difficulties, the veterinarian is responsible for the quality of animal care delivered. Consultant visits are made periodically to assist in care and maintenance of research animals and to take care of health problems that may exist.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Branch's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Neurophysiology, NIMH - - - - -	3580	11.26
Clinical Psychobiology, NIMH - - - - -	3158	9.93
Clinical Science, NIMH - - - - -	2585	8.13
Biological Psychiatry, NIMH - - - - -	2280	7.17
Cerebral Metabolism, NIMH - - - - -	2092	6.58
Neuropharmacology, NIMH - - - - -	2052	6.45
Neurophysiology, NINCDS - - - - -	2025	6.37
Developmental Neurobiology, NICHD - - - - -	2015	6.34
Neuropathology and Neuroanatomical Sciences, NINCDS - - -	1986	6.25
Experimental Therapeutics, NINCDS - - - - -	1875	5.90
General and Comparative Biochemistry, NIMH - - - - -	1668	5.25
Biophysics, NINCDS - - - - -	1498	4.71
Neuropsychology, NIMH - - - - -	1437	4.52
Molecular Biology, NINCDS - - - - -	690	2.17
Brain Evolution and Behavior, NIMH - - - - -	650	2.04
Infectious Diseases, NINCDS - - - - -	596	1.87
Neurochemistry, NIMH - - - - -	507	1.59
Neurochemistry, NINCDS - - - - -	485	1.53
Neuro-Otolaryngology, NINCDS - - - - -	354	1.13
Neural Control, NINCDS - - - - -	259	.81
NIMH (Total)	20,009	62.94
NINCDS (Total)	9,768	30.72
NICHD (Total)*	2,015	6.34
	31,792	100.00

*NICHD loans the Branch one position, and is thus entitled to 1700 hours of service.





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